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LIFE HISTORY OF THE CROWN-GALL ORGANISM IN RELATION TO ITS PATHOGENESIS ON THE RED RASPBERRY[1]

By W. M. BANFIELD[2]

Formerly *agent, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Crown gall, caused by *Phytomonas tumefaciens* (Smith and Town.) Bergey et al. (*Bacterium tumefaciens* Smith and Town.), is a serious and wide-spread disease of cane fruits. Though much attention has been given to various other phases of the crown-gall problem, comparatively little study has been made of it on cane fruits, and the life history of the causal organism in relation to pathogenesis has been but imperfectly understood. The present investigation was undertaken in an effort to define important points in the life history of the causal organism in relation to its pathogenesis on the red raspberry (*Rubus strigosus* Michx.) in the hope that such knowledge might contribute toward the development of control measures.

One strain of *Phytomonas tumefaciens* was used in most of the experiments reported herein. This strain was originally isolated by the writer from a crown gall on Wealthy apple. It has been described as strain 2018 in studies recently reported by Riker et al. (*27*, *28*),[3] and was repeatedly demonstrated throughout the entire course of these investigations to be highly pathogenic on tomato, *Sedum*, tobacco, apple, *Bryophyllum*, sugar beet, and the underground parts of both red and black raspberry varieties. The reactions on several common laboratory media and host plants, induced by 18 strains of the crown-gall organism isolated by the writer from as many collections of crown gall on the roots of red raspberry from Wisconsin, Indiana, and Michigan, have not differed from those induced by strain 2018.

EXIT OF THE CROWN-GALL ORGANISM FROM THE HOST

It has frequently been considered that the crown-gall organism is released from the galls only at the time of their disintegration. From the recent experimental work discussed herein it has been found, however, that under favorable moisture conditions crown-gall bacteria are continuously passed off from the surface of living crown galls.

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[3] Reference is made by number (italic) to Literature Cited, p. 785.

That the soil in which diseased plants are grown contains the crown-gall organism has been suggested by several investigators, who have reported that susceptible plants grown in such soil become diseased (*1, 2, 13, 17, 21, 25, 34, 35, 38*). Patel (*22*) has reported successful isolation of the crown-gall organism from the soil of nursery fields that had produced crops affected by the disease. It has commonly been assumed that the bacteria in the galls are liberated when the galls decay (*5, 6, 17, 18*). The limited experimental evidence does not sustain this widely held assumption. Riker and Keitt (*29*) reported that "from two dozen recorded attempts at isolation from decaying crown-gall tissue, no crown-gall bacteria were found." The present writer has likewise been unsuccessful with either potato-dextrose or bile-agar media (*22*) in many isolation trials from decaying or decayed crown-gall tissue. Robinson and Walkden (*31*) reported that they had observed great numbers of crown-gall bacteria on the surface of living crown galls and that prolonged exposure to running water reduced the bacterial population on the surface by about 200 to 1.

Each of two active crown galls free from decay was successively immersed for different periods in a series of equal volumes of sterile water (table 1). Upon transfer of the gall from one quantity of water to the next in the series, a bacterial plate count was made of the water in which the gall had just been immersed. The results (table 1) are from (1) a crown gall induced on the underground portions of a tomato stem by inoculation about 30 days prior to the time of the experiment, and (2) a crown gall induced by inoculation on the underground portion of the scion of a vigorously growing Wealthy apple graft about 10 weeks prior to the time of the experiment. The bacterial counts made of the successive wash waters show (1) that during the time of the experiment the bacteria were passing from the surface of the gall to the surrounding water, and (2) that the number of bacteria given off to the water during any immersion period was a very small fraction of the total bacterial population of the gall.

TABLE 1.—*Number of crown-gall bacteria found in successive 10 cc volumes of sterile water in which a crown gall had been immersed for the time indicated in the sequence given*

Specimen and treatment	Duration of treatment	Bacteria found in water	Specimen and treatment	Duration of treatment	Bacteria found in water
Tomato crown gall:	*Minutes*	*Number*	Apple crown gall—contd.	*Minutes*	*Number*
Immersed in water [a]	10	4,050,000	Immersed in water [a]	10	2,120,000
Do	30	4,800,000	Do	40	49,670,000
Do	10	697,000	Do	10	893,400
Do	10	2,717,000	Surface washed in running water [c]	70	
Do	30	13,470,000	Immersed in water [a]	10	264,120
Do [b]	10	1,867,000,000	Do	50	19,463,000
Apple crown gall:			Do [b]	10	550,000,000
Immersed in water [a]	10	3,267,000			
Surface washed in running water [c]	35				

[a] Immersed in 10 cc of sterile water in a large test tube.
[b] Macerated and placed in 10 cc of sterile water.
[c] Placed on cheesecloth in a stream of water falling from a faucet.

Aerial portions of the stems of a number of succulent tomato plants were sealed in small cylindrical glass chambers within which the bacterial flora could be controlled. These cylinders were 1¼ inches in

diameter by 3 inches long, with a T side-arm extension of glass tubing one half inch in diameter and three fourths of an inch long. They were slipped down over the leaves of young tomato plants and held in place by clamps supported on ring stands. The open ends of the cylinders were closed by thin sections of a one-hole rubber stopper of suitable diameter. Adhesive tape was bound over the outside of the stoppers and the ends of the cylinders. The ends were then sealed with a mixture of vaseline and paraffin, which was poured in a liquid state through the side arm and around the stem at the bottom of the cylinder. After the mixture had hardened, a thin layer of liquid vaseline was placed on top of the paraffin-vaseline mixture. The top end of the cylinder was sealed by holding the potted plant with the attached cylinder upside down. All motion of the stem within the cylinder that might break the vaseline seal in subsequent manipulations was prevented by binding the pot in which the plant was growing to the base of the ring stand supporting the cylinder (fig. 1).

FIGURE 1.—Glass cylinder sealed over a section of tomato stem. In this manner sections of tomato stem were held for short intervals of time under aseptic conditions.

The surfaces of those portions of the stems which were sealed in the cylinders were then disinfected, inoculated, and again disinfected. Disinfection was accomplished by filling the cylinders for 10 minutes with silver nitrate solution (1: 1,000), introduced, as were all materials subsequently employed, through the side arm of the cylinder, the free end of which was closed by a removable rubber stopper. After the silver nitrate solution had been removed, the cylinders were washed with three successive changes of sterile water, each of which was held in the chamber for 10 minutes.

A plate count was made of a fourth change of sterile water as a check on the effectiveness of the disinfection. One day after disinfection the sterile surfaces of the stems within the cylinders were inoculated by thrusting into each stem a needle that had been smeared

with a crown-gall culture. As protection against contamination, cheesecloth moistened with silver nitrate solution was held over the cylinder during this and other manipulations. Two days after inoculation the stem surfaces within the cylinders were again disinfected, and a plate count similar to that just described was made as a check on the effectiveness of the disinfection.

Two months later an estimate was made of the number of crown-gall bacteria on the surfaces of the stems and large galls induced within the chambers by inoculation. This estimate was obtained by plate counts made of sterile water with which the chamber had been filled and in which the gall had been immersed for 10 minutes. The results from three cylinders in which galls had been successfully induced by the method just described showed that immediately after the first and the second disinfections no crown-gall bacteria were present on the surface of the stem. Moreover, no bacterial or fungal colonies developed in these plates. But 2 months later the plate counts showed 1,000,000, 4,000,000, and 24,000,000 crown-gall bacteria present on the surface of the stems and galls enclosed, respectively, by the three cylinders.

Before disinfection of the gall surfaces, immediately after disinfection, and at various intervals of time thereafter, estimates were made of the bacterial population on the surfaces of three other tomato crown galls held under aseptic conditions within the glass cylinders just described. Before disinfection an average of 3,750,000 living crown-gall bacteria were found. Thirty minutes after disinfection there was none; 4 hours later there were 4,000; 36 hours after disinfection, 13,500; and 5 days after disinfection, 4,400,000.

LONGEVITY AND OVERWINTERING IN SOIL

Observations over a number of years have led to the belief that the crown-gall organism may live for some time in soil in which diseased plants have grown (*1*, *2*, *13*, *21*, *34*, *35*). Experimental evidence reported herein demonstrates that the crown-gall organism overwinters in the soil under ordinary field conditions and that it may live for more than a year in unsterilized soils held free from the growth of seed plants.

Experimental evidence as to the overwintering and longevity of the crown-gall organism has been reported by several writers (*3*, *19*, *23*, *24*). Muncie (*19*) states that tomato plants transplanted to inoculated soil became infected until 154 days after the soil had been inoculated. Patel (*23*) reported that he was able to recover the organism in a pathogenic state from inoculated unsterilized soils in which it had overwintered under field conditions at Ames, Iowa. From a series of isolation trials made from various types of inoculated unsterilized soils held moist under laboratory conditions, he concluded that the organism "may live longer in sandy soils than in clays." He was able successfully to isolate and demonstrate the pathogenicity of the organism 669 days after inoculation in unsterilized loam. On the basis of isolations from the soils of a number of nursery fields, Patel reported that "the pathogenic form of *Ps. tumefaciens* is localized in the field, * * * in close proximity to true galls", and that "organisms resembling

Ps. tumefaciens are not generally found in soils from which crops susceptible to this pathogene are absent."

To determine whether the organism overwinters under field conditions at Madison, Wis., two plots of soil 3 feet square were inoculated September 23, 1926, and isolations were attempted in November and again in May of the following spring. Two diverse soil types were chosen, one a neutral peat and the other a Clyde silt loam. Inoculation to a depth of 2 feet was accomplished by removing the top layer of soil and treating the underlying layers first with a heavy suspension of *Phytomonas tumefaciens*. This was prepared by diluting, at the rate of 1 culture per 6 quarts of water, 6-day mass cultures grown on agar slopes in flat 11-ounce bottles. The suspension was mixed at once with the soil at the rate of 1 pint per cubic foot of soil. Soil samples for isolation were taken from 4 to 7 inches below the surface of the soil in each plot. Soil dilutions of 1:1,000, 1:10,000 and 1:100,000 were used to pour agar plates with Patel's bile medium (*22*). Five plates were poured for each dilution and were incubated 6 days at 28° C. They were then examined for crown-gall-like colonies, a number of which were selected, and with each colony from 2 to 10 inoculations were made on tomato stems. The ability of these bacteria to induce galls when inoculated into susceptible tomato stems was the criterion used in identifying the crown-gall organism. The results, recorded in table 2, show that the organism overwintered in the soil of these two fallow field plots.

TABLE 2.—*Overwintering of Phytomonas tumefaciens in 2 types of soil under field conditions at Madison, Wis., 1926–27* [a]

Date of isolation	*P. tumefaciens* from neutral peat soil			*P. tumefaciens* from Clyde silt-loam soil		
	Colonies tested	Total inoculations	Inoculations positive	Colonies tested	Total inoculations	Inoculations positive
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*
Nov. 12, 1926	20	40	85	20	40	95
May 21, 1927	17	68	68	34	136	15

[a] The soils were inoculated on Sept. 23, 1926.

To gain information as to the length of time the organism might exist in the soil in a pathogenic state, isolations were attempted periodically from two samples of unsterilized inoculated soil. The types used were the neutral-peat and silt-loam soils referred to above. They were held moist and free from the growth of seed plants in open cans in a cellar used for the storage of nursery stock. The isolation technic used and the criterion of successful isolation were the same as in the overwintering studies. The degree of success was measured by the percentages of the inoculations made with these colonies that induced crown galls on tomato stems. From the data recorded in table 3, it is clear that the organism can live for over a year in a pathogenic state in the soil in the absence of seed plants.

When inoculated into tomato stems, the last crown-gall colonies isolated from the silt-loam soil induced but very small galls. Four

TABLE 3.—*Longevity of Phytomonas tumefaciens in samples of 2 unsterilized soils kept in a storage cellar at Madison, Wis., 1926–27* [a]

Date of isolation	*P. tumefaciens* from neutral peat soil			*P. tumefaciens* from Clyde silt-loam soil		
	Colonies tested	Total inoculations	Inoculations positive	Colonies tested	Total inoculations	Inoculations positive
1926	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
May 14	10	20	100	10	20	100
May 31	10	20	100	10	20	100
June 11	10	20	100	10	20	100
June 24	10	20	100	10	20	100
July 25	10	20	100	10	20	80
Sept. 10	10	20	100	10	20	0
Nov. 7	10	20	100	10	20	70
Dec. 7	5	15	100	3	24	29
1927						
Mar. 3	10	40	80	4	24	25
July 2	10	40	70	6	36	0
Oct. 10	5	20	0	1	8	0
Nov. 15	5	20	0			

[a] The soils were inoculated on May 1, 1926, with pure cultures and held under approximately constant moisture conditions at 17° C.

inoculations were made into each of two tomato stems with the three crown-gall-like colonies isolated December 7, 1926. All the inoculations made with two of these colonies were negative. Seven of the eight inoculations made with the third colony produced uniform galls that were, however, very small as compared with the galls induced by parallel inoculations from a 6-day-old transfer of the stock culture with which the soils had been inoculated. Correspondingly, one of the four colonies isolated March 3, 1927, induced relatively small overgrowths on tomato. Although other crown-gall-like colonies were isolated in these and in subsequent trials, none induced any overgrowth response whatsoever in similar pathogenicity tests. These results suggest that the ability of the organism to induce galls on tomato may have declined after it had lived for a long period in the soil; or the small size of the galls induced by the last colonies isolated may have been due to competition with other organisms contained within these colonies (*7*, *27*, *43*).

DISSEMINATION

That crown gall is spread to new areas by the shipment of infected nursery stock has long been known, and careful inspection has often been relied upon to prevent this spread. The writer's experimental evidence discussed herein indicates that rigid inspection of raspberry plants does not prevent the disease from being spread in this way, because incipient stages of infection, which no amount of careful inspection can detect, may be carried by apparently healthy plants.

Selby (*33*, *pp. 111–112*) reported the disease to have occurred in a number of raspberry plantations in the vicinity of Florence, Ohio, and stated that "* * * all the growers procured the plants from the same source. The plantation from which these plants came was found, on examination, to be diseased in the same manner." Lawrence

(*17, p. 17*) 10 years later warned Washington growers against using plants from diseased fields, and said:

* * * even if only those plants are used which do not show signs of the galls the trouble will be quite sure to manifest itself at a later period. The writer has seen this occur in a number of instances.

Bennett (*6, p. 22*) reported:

Plants which have been heeled in or which have been held in cold storage and wet down several times, although they may have few visible galls when planted in the field, have been known to become severely diseased often before the end of the first season. Apparently, in such cases, the bacteria are washed from the few galls which may be in the bundles and find their way into wounds where they become established but do not produce visible swellings till after planting.

Investigators who have studied crown gall have usually found it difficult to obtain plants free from the disease. Thus Smith et al. (*39*) could draw no conclusions from certain inoculation experiments on red raspberry because of the infection that appeared on the roots of the uninoculated control plants. They state that "the infection was probably brought along with them from the nursery, because one or two knots were found on the roots of these plants when they were purchased." Colby's experience, as reported by Keilholz (*16*), in conducting experiments on control of crown gall on raspberries by means of germicides has been similar. He states that conflicting results come

* * * from not knowing whether the plants were clean when planted. An attempt was made to set only clean plants, but it is impossible to detect the crown-gall infection in its incipient stage. If the plant is already infected at planting time, the infection is quite certain to enlarge unless the soil near the plant has been so heavily treated with the germicide that the plant itself is killed.

A test was made by the writer to determine whether apparently healthy red raspberry plants carry incipient stages of infection. Plants of the Latham variety were obtained from a nursery in which the disease was known to be present. These plants were carefully examined for macroscopically visible infections before planting. A number of such plants were found bearing galls 1 mm or more in diameter. These were discarded. Plants selected as not visibly diseased were completely submerged for 20 minutes in a silver nitrate solution (1:1,000) to prevent contamination by crown-gall bacteria that might be present on the surface. They were then set out in beds of soil which had been steamed.

These beds had been heated by the inverted-pan method of high-pressure steaming described by Johnson (*15*). Each bed after being steamed for 30 minutes with a head of 120 pounds pressure, was surrounded by boards as protection against contamination by surface water and left for 1 week before planting. Ten beds thus prepared were planted May 12, 1928, with 600 raspberry plants that had been selected and treated as described.

Twenty-five to fifty raspberry plants were dug and examined at intervals of 2, 3, 11, 12, and 18 months after planting. Of 344 plants harvested from these steamed beds during this period, 51, or 15 percent, were affected with crown gall. An average of 1.7 infections was found on diseased plants harvested within 1 year after planting. The size and condition of the galls found on plants during the first growing season indicated that infection had occurred prior to or soon

after the time of planting. Since it seems improbable that crown-gall organisms in the soil were not killed by the steaming or that the soil was reinfected during the first growing season, it appears that the galls which developed on these plants were due to incipient infections which were present on the plants when received from the nursery but which were not visible. This view is supported by further evidence reported in a later section dealing with (1) the protracted incubation period of crown-gall infections under unfavorable growing conditions and (2) the length of time wounds remain potential avenues of infection by *Phytomonas tumefaciens*. On the raspberry, the disease has been reported by Bennett (*6*) to occur through wounds, and by Dodge and Wilcox (*9*) "through wounds made by insects or by mechanical injuries." The Great Britain Ministry of Agriculture and Fisheries (*12*) very succinctly summarizes the literature on this subject by saying:

> It is generally believed that infection can occur only through wounds, for practically all attempts to produce Crown Gall by inoculation of unwounded tissues have given negative results, whilst the frequent occurrence of Crown Galls on parts of plants which have been cut or wounded strongly supports the view that the organism is a wound parasite. The fact that Crown Galls occur on roots and occasionally on seedlings where no evidence of wounding is apparent does not negative this view, for insignificant wounds made by soil insects or by other means would suffice as ports of entry for the organism.

Observations and experiments have been made by the writer, as previously reported in abstract form (*4*), to determine (1) whether injuries on the underground parts of the red raspberry remain subject to infection for relatively long periods, (2) whether in the absence of injuries to the underground parts of the plant crown-gall infection may occur, and (3) whether root-feeding arthropods are chiefly responsible for the injuries through which crown-gall bacteria gain entrance to the tissues of the plant. The details of this work follow.

LENGTH OF TIME INJURIES ON HOST REMAIN OPEN TO INFECTION

The pronounced susceptibility of the red raspberry to crown-gall infection might be explained in some measure were it demonstrated that injuries on the underground parts of this plant remain open to crown-gall infection for relatively long periods. This phase of the problem was studied in four series of experiments over a period of 3 years. Plants in various stages of development were studied under both field and greenhouse conditions. In both types of experiment all injuries were made at one time and inoculum was applied at various intervals after wounding. The injuries in all cases were made by scraping through the cambial layer with a scalpel. The plants used were grown in soil that had previously been steamed, and precautions were taken against contamination of the injuries by crown-gall bacteria before the inoculum was applied. In experiments on plants in the field, because of the comparative inaccessibility of the roots, most of the injuries were made on the underground portion of the stem. After the soil around the points of injury had been removed, the inoculum was applied to the injuries by means of a camel's-hair brush. Every precaution was taken not to disturb the tissues at the points of injury when the soil was removed and the inoculum applied. Examination of the wounds was made several months after the inoculum was applied to the last set of injuries in the

series. The results of the field studies made in this manner (table 4) indicate that injuries on the underground parts of the red raspberry in some cases may remain open to invasion by the crown-gall organism for 6 weeks.

TABLE 4.—*Length of time during which crown-gall infection was secured through injuries made on stems of red raspberry plants in the opening-bud stage of early spring[a] and in the early autumn[b]*

Period between injury and inoculation (days)	Plants injured	Injuries inoculated[c]	Injuries infected at close of season
		OPENING-BUD STAGE OF EARLY SPRING	
	Number	*Number*	*Percent*
1	7	28	57.1
2	5	20	70.0
6	6	24	54.2
18	7	28	43.0
Controls	15	60	0
		EARLY AUTUMN	
2	21	72	77.8
5	17	66	57.6
9	13	43	16.3
42	[d] 13	44	15.2
Controls	20	60	1.6

[a] The injuries were made on the underground section of the stems of red raspberry plants, just before planting them in steamed soil on May 8, 1928.

[b] The injuries were made Sept. 1, 1928, on the underground sections of the stems of red raspberry plants grown in steamed soil.

[c] A suspension of crown-gall bacteria was applied to the injuries with a camel's-hair brush at various intervals after the time of injury.

[d] These plants were practically in a dormant condition when the inoculum was applied to the injuries. The results were recorded at the close of the following season.

ENTRANCE INTO HOST

"Everything we know about crown gall points to wounds as the usual, if not the only way of infection" (*38*). This view, held by Smith in 1920, has been supported by the observations (*10*, *11*, *17*, *19*, *29*, *32*, *41*, *42*) and experiments of all later investigators.[4] Infection has been obtained experimentally on apple (*19*, *20*, *27*, *29*, *36*, *37*), tomato (*19*, *26*), *Ricinus* (*26*, *30*), geranium (*30*), and grape (*17*, *18*) only through wounds.[5]

Under greenhouse conditions similar studies were made on the roots of red raspberry plants grown in root-observation boxes. These plants had been grown for 1 year in the steamed-soil plots previously described and were free from crown-gall infections when set out in the boxes in the spring, since those that carried latent infections from the nursery had been detected and discarded. The soil in the root-observation boxes was steamed at the beginning of the experiment. In August injuries were made on the roots in the manner already described, and at various periods of time thereafter inoculum was applied to them. Several months after the last group of injuries in

[4] A possible exception is Stapp (*40*), who reported that tumors caused by *Bacterium* (*Phytomonas*) *tumefaciens* occurred at the eyes of potatoes that were planted either in inoculated soil or in ordinary garden soil after being dipped in a suspension of the organism.

[5] Experiments made by various investigators with *Phytomonas rhizogenes* (*28*), often called the apple strain of the crown-gall bacterium, are not included, although recent research (*14*) on this organism shows that it gains entrance to the tissues of its host primarily if not solely through injuries.

the series was treated, all the injuries were examined and the presence or absence of the disease was recorded.

Root-observation boxes of a type similar to those described by Dean (*8*) were used in these experiments. The sides of the boxes were made of wooden hinged doors that covered removable glass plates. The wooden doors could be opened readily and through the glass plates the roots in contact with the glass at the surface of the soil could be observed without disturbing them. The glass plates were removed when the injuries and inoculations were made. The use of these boxes made it possible to manipulate and observe the roots without danger of causing further injury by removing the surrounding soil, as must be done in field experiments. By the middle

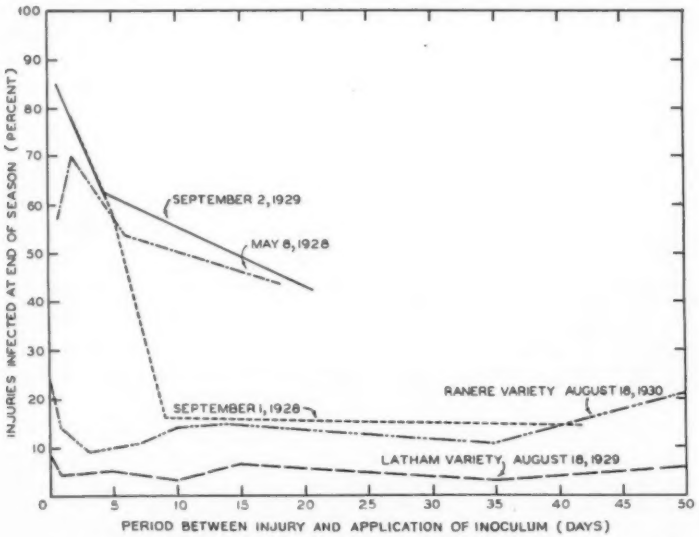

FIGURE 2.—Length of time during which crown-gall infection was secured through wounds on underground parts of red raspberry. The dates given are the dates at which the injuries studied in the experiments were made.

of August the roots in the boxes had grown extensively and a considerable number were in contact with the glass plates at the sides of the boxes.

Injuries were made with a scalpel, as in the field experiments. The injuries were then marked by means of map tacks in the ends of match sticks that were pushed into the soil so that the head of the map tack was adjacent to the injury at the surface of the soil. The injuries in each group in the series were made on the roots of plants in 1 or 2 boxes and inoculation of all the injuries in one group was made at one time, by sprinkling the roots on the surface of the soil with a suspension of crown-gall bacteria. Inoculated boxes were kept separate from those as yet uninoculated, to avoid the danger of contamination of the injuries with crown-gall bacteria before the desired time interval had elapsed. The results of the experiment are shown

in table 5. This experiment was repeated under like conditions the following season, and the time was extended to 50 days. Essentially the same result was obtained as in all earlier experiments (lowest curves, fig. 2).

TABLE 5.—*Length of time during which crown-gall infection was secured through injuries made on roots of red raspberry plants in early autumn,* [a] *1929*

Treatment and date	Period between injury and inoculation	Injuries treated	Injuries infected	
			Oct. 3	Nov. 26
	Days	*Number*	*Percent*	*Percent*
Injuries inoculated: [b]				
Sept. 3	1	40	75	85
Sept. 6	4	50	40	64
Sept. 23	21	50	0	42
No inoculum applied		100	0	0
Control injuries made and inoculated:				
Sept. 3	0	10	70	90
Sept. 6	0	10	60	90
Sept. 23	0	10	0	70
Oct. 4	0	31	[c] 10	71
Control needle-thrust inoculations:				
Aug. 22–Sept. 3	0	90	87	96
Oct. 4	0	31	[c] 7	77

[a] The injuries were made on Sept. 2 on the roots of healthy crown-gall-free plants grown in steamed soil in root-observation boxes.
[b] A suspension of crown-gall bacteria was sprayed over the root and the soil in the root-observation boxes.
[c] Observed on Oct. 24.

The data obtained from each experiment are presented in figure 2. The curves show that the percentage of treated injuries that became infected decreased as the interval between the time of injury and the application of inoculum increased. On vigorous plants this decrease was at first considerable, but after 10 days to 3 weeks the percentage of infected injuries tended to remain constant, indicating probably that a fair proportion of such injuries were rapidly closed to infection but that a small part of the injuries remained subject to infection for 3 to 7 weeks or longer.

A study was made to determine whether injuries on the underground parts of a variety more resistant to crown gall would be subject to invasion by the parasite for a shorter time. Latham and the supposedly more resistant variety Ranere were used. The experiment was carried out on each variety under the same conditions and in the same manner as the experiments described above. The results are recorded in figure 2. The erratic results obtained in the 1930 season may have been due in part to the low vitality of the plants used, a condition that probably resulted from late planting. The results indicate, as did those of all earlier experiments, that injuries to the underground portion of the Latham red raspberry remain subject to crown-gall infection for a considerable period. The comparative record on the more resistant variety, Ranere, suggests that in this respect there is little difference in these red raspberry varieties.

Ranere, often called St. Regis, has been reported resistant and Cuthbert susceptible to crown gall. Comparative studies by the present writer of the development of crown gall in five varieties of raspberry grown during the 1930 season in inoculated sandy-loam soil indicate that Ranere displays some resistance to the disease (table 6). In this experiment the root systems of the Cuthbert plants used were

but one third as extensive as those of the Ranere. Had the plants been equal in size the difference between the two varieties in all probability would have been more sharply defined. The Latham variety was not intentionally used in these tests. A few plants developed from roots left in these plots from an earlier planting.

TABLE 6.—*Development of crown gall on 5 varieties of raspberry grown in inoculated soil, 1930*

Variety	Total plants grown	Average galls per plant	Plants infected	Plants visibly injured by root-feeding arthropods
	Number	*Number*	*Percent*	*Percent*
Cuthbert	90	7.0	98	62
Cumberland	52	6.6	94	85
Latham	8	6.6	88	75
Plum Farmer	48	4.9	75	77
Ranere	84	3.2	72	39

Injuries are inevitably caused to the root systems of raspberry plants in storage and in transit. To determine whether injuries to the roots caused prior to planting are subject to infection, 12 plants received from a nursery were injured just before they were planted in the inoculated soil plots described under the heading Seasonal development (p. 779). The injuries were marked by loosely encircling the stem or root at the point of injury with an aluminum wire band. Inspection 14 weeks after planting showed that 19 of the 48 injuries (40 percent) so treated had become infected. Of the 71 injuries made on plants set out in plots of soil that had been steamed none became infected.

INFLUENCE OF MECHANICAL INJURY

Experiments were made to determine whether crown-gall bacteria enter the red raspberry only through injuries. Under controlled conditions in the greenhouse experiments, crown-gall infection did not occur in the absence of either of two factors; namely, (1) injuries to the underground parts of the plant and (2) crown-gall bacteria in the soil in which the plants were grown. Of plants grown in inoculated soil and not guarded against injuries to their underground parts, 80 percent developed the disease. The average number of infections on these diseased plants under controlled conditions was about equal to that found for diseased plants grown in inoculated soil in the field.

These experiments were carried out in the greenhouse on a limited number of plants known to be practically free from crown-gall infections. The plants were disinfected by immersion in silver nitrate solution (1:1,000) for 20 minutes and then planted in 10- or 12-inch pots or in root-observation boxes in soil that had previously been steamed. Prior to the time of the experiment the plants had been grown for 1 or 2 years in the steamed-soil plots previously described. During this period the incipient stages of infection developed that were carried on the plants from the nursery. By discarding the infected plants and selecting only those that had grown at a distance of 3 feet or more from them, plants were obtained having a minimum of incipient macroscopically nonvisible infections. The extent of this minimum quantity of infection was ascertained at the close of each

experiment by recording the number of infections found on plants that had not been exposed to crown-gall bacteria during the course of the experiment. Tables 7 and 8 show that no infections were present on the plants selected 1 year after planting in steamed soil, but that a small number of such infections were present on plants removed at the end of 2 years from the steamed-soil beds.

The plants were guarded against the effects of mechanical injury of two types: (1) Injury received when the plants were transplanted at the beginning of the experiments and (2) injury caused by root-feeding arthropods. The injury received in transplanting was practically eliminated as a factor in infection by holding the steam-sterilized soil in which these plants were grown free from crown-gall bacteria for 3 or 4 months. A few injuries received in transplanting became infected when the soil was inoculated 3.5 months after the time of transplanting. Practically, however, this procedure furnished an adequate control against this kind of infection. Injury caused by root-feeding arthropods was guarded against in the 1927–29 experiments by setting the pots or boxes on planks held 3 feet above the greenhouse floor by wooden supports 4 inches square. The boxes or pots were held three fourths of an inch above the surface of the planks by wooden supports 1 by 2 inches. In the experiments conducted in the 1930 season, protection against arthropods was attained by steam sterilization of the soil used, disinfection of plants and containers, and enclosing the plants and their containers within cheese-cloth cages. The plants that were not guarded against root-feeding arthropods were held on the ground (covered by a dense growth of weeds and sod) outside the greenhouse or on the soil floor within the greenhouse.

In the experimental procedure the three main groups of plants were subjected to the variations in conditions shown in tables 7 and 8.

TABLE 7.—*Influence of (1) injuries caused chiefly by root-feeding arthropods and (2) the presence of crown-gall bacteria in the soil on crown-gall infection of red raspberry, 1927–29*

Total plants	Injuries caused by—		Crown-gall bacteria in soil	Infections per plant (average number)
	Transplanting	Root-feeding arthropods		
36	Present	Present	Present	6.1
18	do	do	None	.0
25	None	None	Present	.2

TABLE 8.—*Influence of injuries caused chiefly by root-feeding arthropods on development of crown gall on red raspberry plants grown in inoculated soil in 1930*

Plants grown	Conditions of growth		Injuries caused by		Crown-gall bacteria	Galls per plant (average number)
	Place	Time of transplanting	Transplanting	Root-feeding arthropods		
274	Field	Early [a]	Present	Present	Present	4.8
47	do	Late [b]	do	do	do	2.5
27	Greenhouse	do	do	do	do	2.4
39	do	do	do	do	None	.3
91	do	do	None	None	Present	.2

[a] For a more detailed record of these plants see table 6.

[b] Latham plants were used in all the greenhouse series and in the group of plants transplanted late in the field.

The results of the first series of experiments made in the seasons of 1927 to 1929 are summarized in table 7. Infection was present only on those plants subjected to both injury and the crown-gall bacterium. Four infections occurred on plants subjected to inoculum and guarded against mechanical injury caused by arthropods and against the effect of injury received in transplanting. These galls were 3 mm or less in diameter when the plants were harvested, and occurred at points of contact of root surface and the glass sides of the root-observation boxes used in the experiment. It is probable that these infections took place through minute abrasions of the root surface caused by sand grains. When pressure was applied to the doors covering the glass sides of the boxes, sand grains might have been forced into the roots at these points of contact. Infection occurred in 90 percent of the injuries caused for control purposes on the underground parts of these plants just prior to the inoculation of the soil. Of 201 injuries caused to these plants at various intervals up to 21 days prior to the application of inoculum to the soil, 134 were galled at the close of the growing season. From the ready response of the plants to inoculation and of the injuries caused on these plants to infection (table 5) it is apparent that the lack of infection at other points than those intentionally injured or inoculated could not be attributed to the late application of inoculum to the soil in which the plants were grown. The results of these experiments indicate that there are no natural openings through which infection takes place in early autumn on the underground parts of the red raspberry.

These experiments were repeated on a larger number of plants in 1930. The methods used and the results obtained were essentially the same (table 8). The first set of plants used in this experiment was stunted by lack of adequate drainage in the new type of root-observation box used, and a second series was planted in the same boxes in early June after this defect in construction had been corrected. The smaller amount of infection on the plants in this series subjected to both injury and crown-gall bacteria is believed to have been due to the lower vitality of the plants, which resulted from late transplanting. Under field conditions in inoculated soil the amount of infection that occurred on a number of the same lot of plants was practically equal to that found on the plants exposed to inoculum and arthropods in the controlled series as previously described.

INFLUENCE OF ROOT-FEEDING ARTHROPODS

Many observations suggest that root-feeding arthropods cause most of the injury to the underground parts of the raspberry through which crown-gall infection occurs. Injuries caused by such arthropods have been found in every planting of raspberries studied by the writer at Madison, Wis. In some plantings 85 percent of the plants were visibly injured in this way. Crown-gall infection frequently occurred at such points of injury to the underground parts of plants grown in inoculated soil, but most of the injuries did not become infected. The injury appeared to be caused by a number of root-feeding arthropod forms.

The injury found on the plants was of several types, ranging from very minute abrasions of the surface of delicate rootlets to severe pruning of lateral roots at the crown of the plant and decortication of

the underground stem and larger roots. The more insignificant types of injury appeared on tiny roots and could be detected with the naked eye only by the most minute and careful examination (fig. 3, *A*). On the smaller roots (2 mm or less in diameter) cortical incisions were frequently abundant. These occurred singly (fig. 3, *L*) or in series close together and appeared as dark brown parallel lines at right angles to the longitudinal axis of the root (fig. 3, *I*, *J*, *N*). Occasionally the smaller roots were decorticated for short distances (fig. 3, *K*) or had small shallow cavities gouged out of their surfaces (fig. 3, *E*, *F*, *G*, *M*, *O*). These lesser injuries were characteristic of those found on plants grown on the low reclaimed soil of the marsh described in an earlier section of this paper. The most conspicuous type of injury was found on plants grown on the well-drained sandy-loam plots and consisted of large shallow cavities gouged out of the larger roots and the underground portions of the stems (fig. 4, *A* to *E*). Besides such injury, many branch roots, often 50 percent or more, were completely severed from the plant (fig. 4, *F*). This type of injury was not observed during the seasons of 1928 and 1929. The majority of the plants grown on the sandy-loam soil in 1930 were injured in this manner (table 6).

Crown-gall infection developed on a large percentage of injuries to the underground parts of red raspberrry plants grown in inoculated soil (figs. 3 and 4). Usually after several months of development the galls had completely obscured the injury through which the infection took place. Gall development obscured within a few weeks the smaller injuries through which infection occurred (fig. 3, *A* to *H*, *N* to *S*). In the seasonal-development study discussed later there was no indication of the manner in which infection occurred in over 95 percent of the galls. The injuries through which infection entered the plants grown in the reclaimed marsh soil were of an inconspicuous type (fig.3).

In two types of experiment, involving the exclusion and inclusion of arthropods, respectively, it has been shown that root-feeding arthropods cause the injuries through which crown-gall infection occurs on the raspberry. In the exclusion experiments, groups of Latham red raspberry plants were grown in inoculated soil from some of which arthropods were excluded and from some of which they were not excluded. The methods have been already described. When no precautions were taken to exclude root-feeding arthropods from these plants, infection occurred as abundantly as under the usual field conditions. When the plants were protected from such arthropods in the soil in which they were grown no infection was observed (tables 7 and 8). The field observations and experimental results indicate that under ordinary conditions root-feeding arthropods are the chief factor in creating the injuries through which crown-gall infections occur. In these experiments no attempt was made to ascertain what arthropod species caused the injuries in question.

The arthropod-inclusion experiments consisted in confining root-feeding species (1) to limited areas of root surface and (2) to the soil in the container in which the plants were grown. The arthropod forms used were click-beetle larvae (Elateridae), millepedes (Diplopoda), and white grubs (*Phyllophaga* sp.). In the first type of experiment the larvae were confined to short uninjured lengths of lateral roots from 1 to 3 mm in diameter, by enclosing a length of 2 to

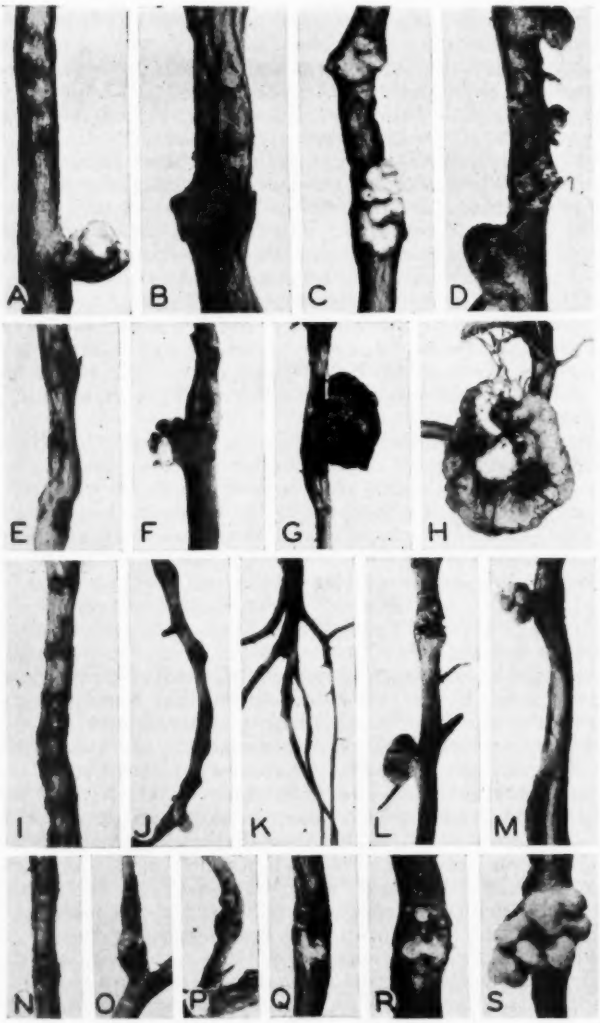

FIGURE 3.—Representative injuries caused by root-feeding arthropods on roots of Latham red raspberry plants grown in the field in soil inoculated with *Phytomonas tumefaciens*. Crown-gall infection was found commonly on these plants in association with injuries caused by root-feeding arthropods. Crown gall has developed from infection through a number of injuries shown. Injuries: *A*, Minute abrasions of the root cortex (upper half of figure) at top of stem, barely visible to the naked eye; *B*, *C*, *E*, *F*, *G*, *M*, *O*, *P*, small shallow cavities chewed from the roots; *I*, *J*, *N*, multiple incisions in the roots; *K*, decorticated small lateral roots. Infections: *B*, *K*, *Q*, Crown-gall overgrowth just appearing from infection through injuries; *C*, *G*, *O*, *R*, crown-gall overgrowth is rapidly obscuring the injuries through which infection occurred; *D*, *H*, *L*, *S*, lower *A*, *F*, *J*, upper *M*, crown-gall overgrowth has obscured the injury through which infection occurred; *Q*, *R*, *S*, stages of crown-gall formation after infection through injuries caused by a scalpel; *H*, circular depressions have resulted from the feeding of grubs on a crown gall. Scale of enlargement: *K*, × 1; *H*, *Q*, *R*, *S*, × 2; *C*, *D*, × 3; *G*, *I*, *J*, *O*, *P*, × 4; *B*, *E*, *F*, *M*, *N*, × 5; *A*, *L*, × 7.

4 inches of one or several such roots in a wire screen. The wire screen used was of brass (100 meshes per square inch), and was sewed with brass wire in the form of a tight sack, 4 by 3 by 1.5 inches, about the

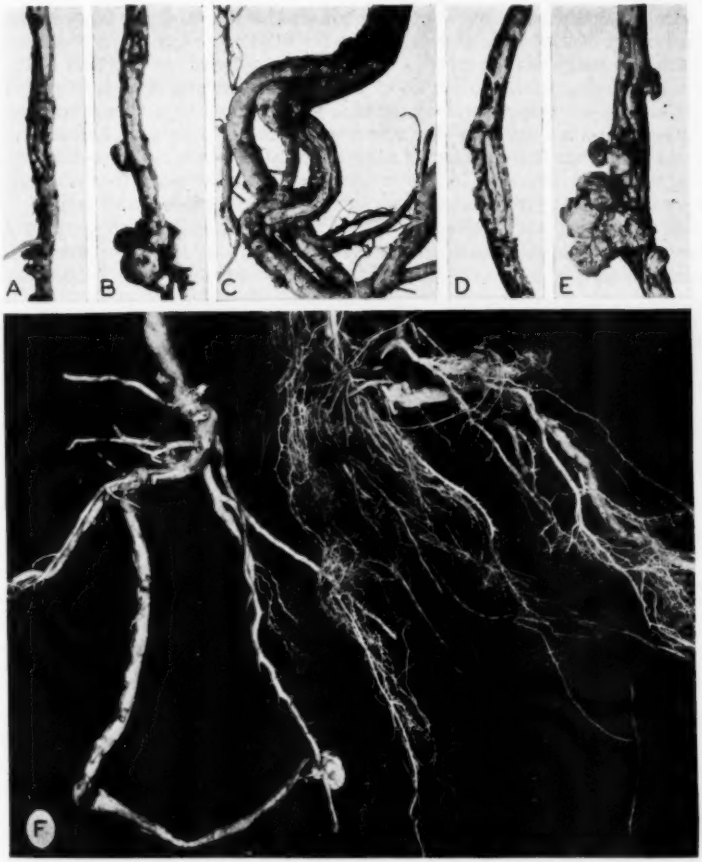

FIGURE 4.—Typical white-grub injury on the roots of raspberry plants. Crown-gall infections were usually associated with this type of injury in 1930 at Madison, Wis., on all raspberry plants grown in the field in soil inoculated with *Phytomonas tumefaciens*. Injuries: Shallow to deep cavities were gouged by white-grubs, *Phyllophaga* sp., from the larger roots, and the underground portions of the stem of both red (*A, B, F*) and black (*C, D, E*) raspberry varieties. Frequently 50 percent or more (*F*, left) of the lateral roots were eaten from plants in field plots. *A, B*, Injuries caused by grubs on the roots of Latham red raspberry plants grown in a container of inoculated soil in which white grubs had been placed. Infections; *B*, Crown-gall development at top not perceptible from infections through injury; *A, C*, Barely perceptible at the injury; *D*, rapidly covering the injury; and *B, E*, obscuring the injury. *F*, Representative root system of a series of plants grown in the greenhouse in pots of inoculated soil and subjected to the following conditions: White grubs were placed in the soil (left) and root-feeding arthropods were excluded from the soil (right). Crown-gall infection has occurred through several of the injuries caused by the grubs. No injuries and no infection occurred on plants from which arthropods were excluded. Scale of enlargement: *A, B, D*, and *E*, × 2; *C*, natural size; *F*, ½ natural size.

roots. The smaller spaces around the points where the roots protruded from the sack were stuffed with glass wool. Two wireworms were put into each of two such wire-screen sacks, which were then

filled with soil and sewed shut. One millepede was put into each of two other screen cages. One white grub was put into each of four similar root enclosures. The soil in the sacks was then inoculated by applying to the soil of the root-observation boxes used a suspension of crown-gall bacteria. Six weeks later the screen cages were removed and observations were made. The roots in one of the wireworm cages were not noticeably injured. In the other, one side of the root enclosed was decorticated for a length of 2 inches and crown-gall infection had developed at two points along this area of injury. Since the injury extended past the end of the screen cage, it is possible that it may have been made in fastening the cage about the root. The millepede in one cage had decorticated the root in two places, no infection had occurred, and the root had died. The white grubs had eaten all the roots enclosed within each of the four screen cages in which they had been confined, leaving not a trace. Of the four, two escaped into the soil in the free space of the root-observation box. Examination of the roots in the box revealed injuries identical in type with those found on the majority of plants grown in the field in 1930 (fig. 4, *A*, *B*). Crown-gall infection had occurred through two of seven such injuries. All the arthropods employed in the foregoing studies, except one millepede, were recovered alive at the close of the study.

A further experiment was made in which white grubs were placed at large in pots in which disease-free raspberry plants were growing. The soil was then inoculated, as was the soil in which a control group of plants were growing. The control group was protected from all arthropod forms. In each group there were ten 6-inch pots, each containing two plants. Six weeks after two large white grubs had been placed in each of the pots of the first group and the soil of both groups had been inoculated with a suspension of crown-gall bacteria, all plants were washed free of soil and examined. Of the 20 plants grown in pots in which grubs had been placed, all were conspicuously injured. Large shallow cavities had been gouged from the underground part of the stem and the larger roots. Frequently the roots had been either chewed completely off or about half eaten away for a length of 1 to several inches. The lateral roots of smaller diameter had been largely eaten away (fig. 4, *F*). Of these 20 plants 7 had developed crown-gall infection at the points of white-grub injury, with an average of 3.6 galls per plant; 2 of the remaining 13 had died. No injuries were found on the underground parts of the plants in the parallel group that had been protected from all arthropod forms, and no crown gall developed on these, though both groups had been exposed alike to inoculum. The injury caused by these grubs was identical with that found on plants grown in the inoculated field plots in which white grubs were found in abundance (fig. 4, *B* to *E*; table 6). From these experiments and observations it is concluded that in 1930 white grubs caused most of the injury through which crown-gall infection developed on raspberry plants grown in inoculated soil at Madison, Wis.

INCUBATION PERIODS

During the studies, already described, on the length of time wounds remain open to infection, variation in the incubation period of crown-gall infections on the underground parts of red-raspberry plants was

observed to occur. This variation appeared to be correlated with (1) the general condition of the host plant in response to environmental factors, particularly those relating to the season, and (2) local variation within individual plants due to differences in age and vigor of roots. It was observed that overgrowths of appreciable size appeared within 11 days after inoculations made on August 23 (table 5), whereas inoculations made on October 4 did not induce appreciable overgrowths until 3 weeks later. From the data recorded in table 5 it is clear that many inoculations which had not induced macroscopically visible overgrowths 3 to 4 weeks after the application of inoculum developed such overgrowths later. In these studies the incubation period of crown-gall infections on the roots of the red raspberry was observed to range from 11 days to more than 4 weeks.

A knowledge of the prolonged incubation period, which appears to lengthen as the plants approach the dormant condition, and of the relatively long period in which injuries to the underground parts of the red raspberry may remain subject to infection by crown-gall bacteria, is important in understanding the incipient stages of infection by which the disease is disseminated to new areas.

SEASONAL DEVELOPMENT

Little has been reported heretofore concerning the time at which crown-gall infection occurs, and the course of the development of this disease on the red raspberry has remained obscure. From seasonal development studies reported herein it was found that crown-gall infection occurred (1) at random on all underground parts of the plants and (2) at a practically uniform rate throughout the course of the two seasons studied.

For a period of 2 years a study was made of the seasonal development of crown gall in a planting of Latham red raspberries. All the plants studied were without macroscopically visible infections when they were planted in a field plot of inoculated Clyde silt-loam soil. The plot was inoculated 1 day before planting by mixing into the upper 9 inches of soil a suspension of crown-gall bacteria applied at the rate of 1 pint per square foot of soil. The suspension used was made and applied as described earlier for the overwintering studies. From time to time during the season in which these plants were set out and during the following season a number of plants were dug at random from the plot and their condition with regard to the disease was recorded. Each plant was washed free of soil particles in a stream of water, and all its parts were then examined minutely for macroscopically visible infections. In this way all visible galls on the plant were recorded with notations as to their size and position. From 40 to 75 plants were examined and discarded at each observation. The data obtained from this study are presented in condensed form in tables 9, 10, and 11, and in figure 5.

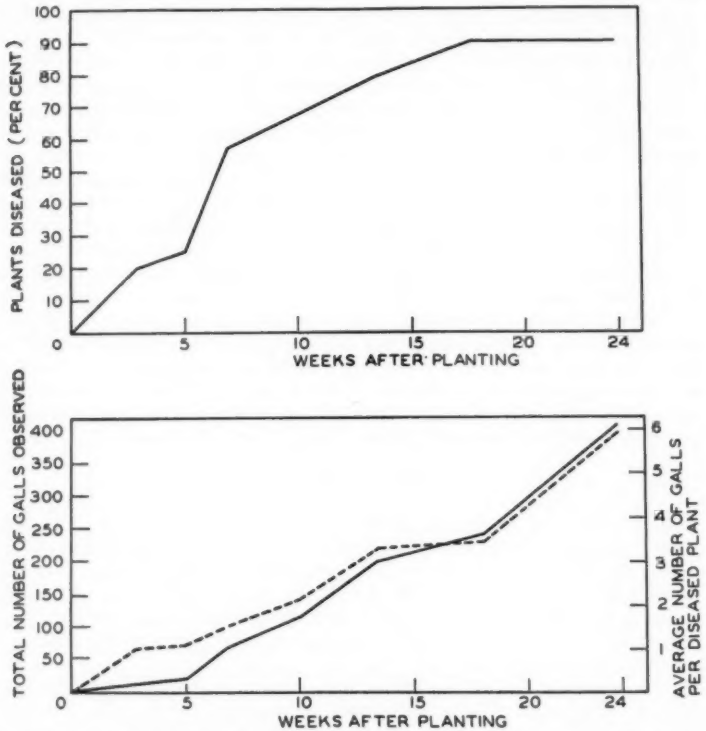
PLANTS DISEASED (PER CENT)
WEEKS AFTER PLANTING
TOTAL NUMBER OF GALLS OBSERVED
AVERAGE NUMBER OF GALLS PER DISEASED PLANT
WEEKS AFTER PLANTING

FIGURE 5.—Seasonal development of crown gall on underground parts of red raspberry plants grown in the field in soil inoculated with the crown-gall organism. Broken line in lower graph indicates average number of galls per diseased plant.

TABLE 9.—*Seasonal development of crown gall on underground parts of red raspberry plants grown in inoculated soil, 1928–29*

Date	Length of time after planting	Plants examined		Galls present					
		Total	Infected	Total	Average per infected plant	Distribution according to indicated diameter			
						0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Larger than 10 mm
1928	*Weeks*	*Number*	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
June 6	3	40	20	8	1.0	3	2	3	0
June 23	5	40	25	11	1.1	8	1	2	0
July 6	7	75	56	68	1.6	49	8	7	6
July 27	10	40	68	61	2.2	28	15	11	7
Aug. 17	13	75	80	218	3.6	54	67	68	29
Sept. 17	18	75	91	247	3.6	36	83	61	67
Oct 28	24	75	92	402	5.8	66	99	105	132
1929									
June 1	55	56	90	285	6.3	34	59	133	43
Aug. 1	64	50	94	* 271	5.7	3	54	110	34
Nov. 1	78	50	96	302	6.3	12	24	180	86

* The total number of galls here includes 70 which had disintegrated to the stage where they could be detected only by scars left on the roots.

TABLE 10.—*Size and location of crown galls observed during the first season on underground parts of red raspberry plants grown in inoculated soil, 1928* [a]

Length of time after planting (weeks)	Location and size of crown galls											
	Galls of indicated diameter on stem				Galls of indicated diameter on branch roots larger than 2 mm				Galls of indicated diameter on branch roots less than 2 mm			
	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Greater than 10 mm	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Greater than 10 mm	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Greater than 10 mm
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
3	2	2	2	0	1	0	1	0	0	0	0	0
5	2	1	2	0	2	0	0	0	4	0	0	0
7	6	2	3	3	2	4	3	2	41	2	1	0
10	0	1	5	4	7	11	4	3	21	3	2	0
13	2	10	17	10	21	20	25	15	31	37	26	4
18	0	11	14	19	6	24	25	43	30	48	22	5
24	4	12	10	39	16	37	64	74	46	50	31	19

[a] For dates of observation, number of plants examined, number of infected plants, and total number of galls observed, see table 9.

TABLE 11.—*Size and condition of crown galls observed during the second season on underground parts of red raspberry plants grown in inoculated soil, 1929* [a]

Date of observation	Condition of galls observed	Total galls observed	Average galls per plant	Distribution of galls according to diameter			
				0.5 to 2 mm	2.1 to 5 mm	5.1 to 10 mm	Larger than 10 mm
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
June 1	Living	132	2.9	28	34	53	17
	Dead	153	3.4	6	25	78	44
Aug. 1	Living	123	2.6	2	41	57	23
	Dead	[b] 148	3.1	1	13	53	11
Nov. 1	Living	170	3.5	12	19	107	32
	Dead	132	2.8	0	5	73	54

[a] The number of plants examined, number of diseased plants, and the average number of infections per plant are given in table 9.

[b] 70 galls which had disintegrated to the stage where their presence could be distinguished only by the scars that their decay had left on the roots could not be recorded as to size.

As an ecological check, two smaller plots of Miami sandy loam were inoculated and planted at the same time as the silt-loam plot described above. These smaller plots were situated on a gentle slope about 1 mile distant from the drained low marshland on which the silt-loam plot was located. The same soil treatments and the same plant materials were used in both experiments. The plants in the smaller plots were not examined until the close of the second season, however, at which time all were harvested and a detailed record was made of their condition with reference to the disease. The comparative record of the development of crown gall on plants grown in these inoculated sandy-loam and silt-loam soils is shown in table 12.

TABLE 12.—*Comparison of crown galls found on red raspberry plants grown in sandy-loam and in clay-loam inoculated soils* [a]

Age of plants and soil type	Plot no.	Total plants	Infected plants	Total galls	Average galls per infected plant	Distribution according to diameter of crown galls observed							
						Living				Dead			
						0.5 to 2 mm	2.1 to 5 mm	5.1 to 10mm	Larger than 10 mm	0.5 to 2 mm	2.1 to 5 mm	5.1 to 10mm	Larger than 10 mm
2-year plants:		*No.*	*Pct.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Sandy loam	22	37	95	285	8.1	32	28	94	31	0	2	49	49
Do	21	34	94	[b] 283	8.8	51	53	118	61				
Clay loam	M	50	96	302	6.3	12	19	107	32	0	5	73	54
1-year plants:													
Sandy loam	22	28	57	35	2.2	14	6	9	6	0	0	0	0
Do	21	36	67	[c] 131	5.4								
Clay loam	M	94	17	22	1.4	3	2	13	4	0	0	0	0

[a] Data were recorded at time of harvesting the plants at close of second growing season subsequent to planting in inoculated soil.
[b] No record was made of the condition of these galls.
[c] No record was made of the condition or size of these galls.

A further check to ascertain the number of undeveloped infections that these plants carried when shipped from commercial nursery storage was made in parallel plantings on sandy-loam plots that had been steamed. These plots were at a slightly higher level and were adjacent to the inoculated sandy-loam plots described above. The development of the disease on the plants in these steamed plots has been discussed already. All plots were planted from a shipment of Latham red raspberry plants received from a nursery in May 1928.

The following is a summary and brief discussion of the results of the study of the seasonal development of crown gall on red raspberry plants grown in inoculated soils.

Infection occurred at all times and at a practically uniform rate throughout the entire growing season.

The number of plants showing crown-gall infection increased rapidly from 20 percent in early June of the first season (an increase at that time of 5 percent over the controls planted in the steamed beds) to about 90 percent by the middle of September. The number of diseased plants then gradually increased to 96 percent by the close of the second season (table 9, fig. 5).

The number of infections per plant increased steadily and at a practically uniform rate throughout the growing season. This increase appears as a relatively uniform progression when the number of infections is plotted against time (fig. 5). The average number of infections found per diseased plant in June was 1.0 as contrasted with 5.8 at the close of the season. In June of the second season a maximum of 6.3 infections per diseased plant was reached. Although new infections continually appeared during the second season (table 11) the number of galls found per diseased plant remained about constant. This may have been due in part to decomposition of galls formed as a result of infections that occurred during the first season.

Infection appeared to occur at random on all underground parts (fig. 6). It did not seem to be confined to any particular developmental phase or portion of the plant. On the underground section of the stem, on the branch roots of large or small diameter, infection

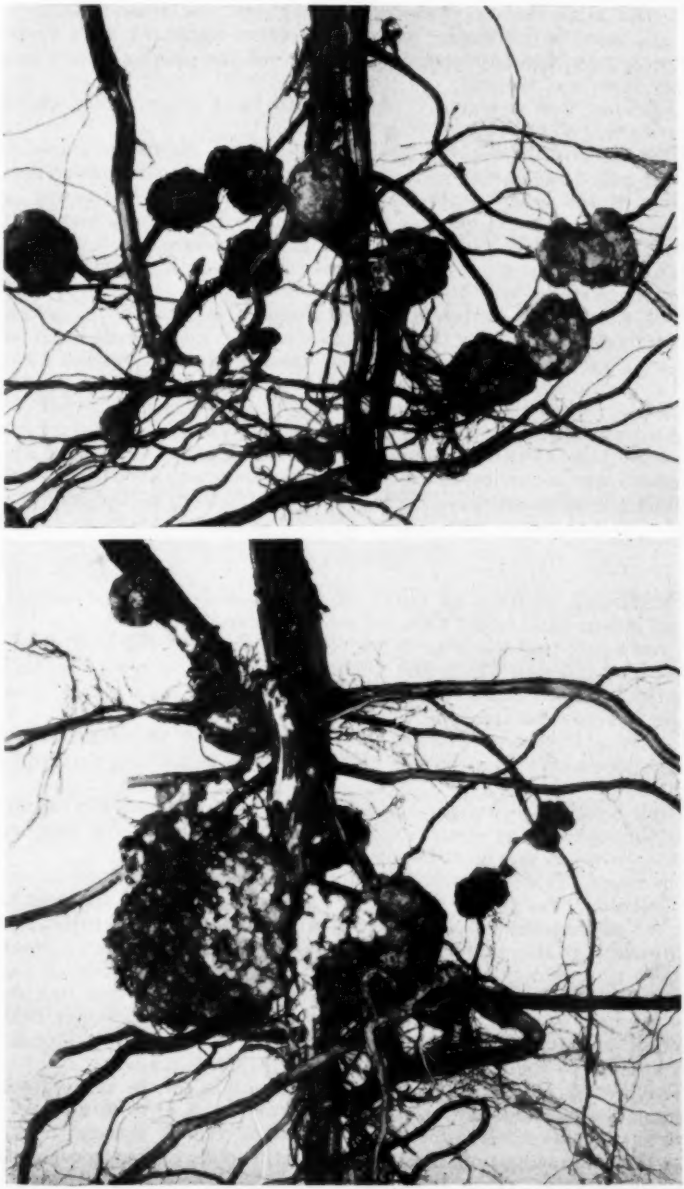

FIGURE 6.—Typical crown gall on the roots of Latham red raspberry plants grown in the field in soil inoculated with *Phytomonas tumefaciens*. This type of crown gall occurred on the roots of over 95 percent of the plants grown under these conditions. Aerial portions of the plants were affected very rarely—less than 0.2 percent of all plants grown.

occurred alike and continuously throughout the season (table 10). As discussed in an earlier section, infection appeared with greatest frequency on those underground portions of the plant within 4 inches of the surface of the soil.

Infection was repeatedly observed to have taken place through injuries caused by root-feeding arthropods.

The disease developed more extensively on plants grown on the higher well-drained sandy loam (table 12) than on plants grown on the reclaimed silt loam of the marsh. At the time of harvesting, the disease seemed from general observation to be more severe on the plants grown in the sandy loam. That the disease did develop more extensively on the plants in these plots is apparent from the comparative record (table 12), from which it is shown that:

The number of first-year plants diseased was 57 to 67 percent, respectively, for the sandy-loam plots and 17 for the silt-loam plot; the average number of infections per diseased first-year plant was 2.2 and 5.4, respectively, for the sandy-loam plots, and 1.4 for the silt-loam plot; the average number of infections per diseased 2-year plant was 8.1 and 8.8, respectively, for the sandy-loam plots and 6.3 for the silt-loam plot; toward the end of the second season the rate at which infection was occurring in the sandy-loam plots was about three times that in the silt-loam plot. This rate is a function of the number of 0.5 to 2.0 mm galls found on the plants at any observation.

SUMMARY

Crown-gall bacteria are given off continuously from the surface of living crown galls under suitable moisture conditions.

Crown-gall bacteria were found to overwinter in the soil of fallow fields at Madison, Wis., and to exist in a pathogenic state for 14 months in unsterilized soil held free from the growth of seed plants under nursery storage conditions.

Crown gall is carried from the nursery to new areas as incipient undeveloped infections on the raspberry that cannot be detected by inspection.

Crown-gall infection was found to occur at a practically uniform rate throughout the course of the growing season on red raspberry plants grown in inoculated soil.

Crown-gall infection occurs on the raspberry only through injuries. No infection was found on plants which were exposed to inoculum but the underground parts of which were protected from injury.

Injuries on the underground parts of the red raspberry remain subject to crown-gall infection for a relatively long period. A large percentage of the injuries studied became infected when inoculum was applied to their surfaces 3 weeks after the injury was made. A small percentage of those studied became infected when inoculum was applied to them 7 weeks after the injury was made.

The incubation period of crown-gall infection on the underground parts of the red raspberry was found to vary from 11 to more than 28 days as a result of environmental conditions, chiefly seasonal.

In these investigations root-feeding arthropods caused most of the injuries through which crown-gall bacteria entered. The injuries found on the underground parts of the plants probably were caused by a number of species of arthropods. White grubs caused most of the injuries through which infection occurred in 1930.

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CHROMOSOMES IN HYBRIDS BETWEEN EUCHLAENA PERENNIS AND ZEA MAYS[1]

By A. E. LONGLEY[2]

Associate botanist, Division of Genetics and Biophysics, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

Hybrids between maize (*Zea mays* L.) and perennial teosinte (*Euchlaena perennis* Hitchc.) offer a favorable opportunity to follow the behavior of triplicate sets of chromosomes in a triploid hybrid. Maize, the diploid parent, has been carefully analyzed both genetically and cytologically. Perennial teosinte seems to be unquestionably a true tetraploid. Its tetraploid character is evident from (1) the prevalence of tetravalent chromosomes at diakinesis observed by Randolph [3] and by the writer; (2) the X-ray experiments of Randolph (*17*),[4] who obtained from the 20-chromosome annual *Euchlaena* a 40-chromosome perennial plant very like *E. perennis*; and (3) genetic studies which show that F_1 teosinte-maize hybrids contain three sets of homologous chromosomes. Although extensive genetic studies were carried on in conjunction with the cytological studies, the present paper treats only of the chromosomes as found in F_1 and later hybrid generations.

The first plants of perennial teosinte were found in Mexico by Hitchcock in 1910 and were introduced into this country by Collins (*4*) in 1921. The first hybrid with maize was made early in 1922 by C. G. Marshall. The breeding has been done largely at the United States San Diego Acclimatization Garden at Torrey Pines, Calif., where the more promising perennial hybrid forms are maintained in permanent plantings. The methods of crossing have been similar to those used in corn-hybridizing studies, but to bring plants with very different flowering dates into flower at the same time, plantings were made at different seasons and late-flowering plants were frequently given an artificial short day.

The cytological material, which had been collected just before the tassel emerged from the leaves, was put in Carnoy's killing fluid for half an hour; this fluid was then poured off and the material was covered with absolute alcohol. Material preserved in this way is generally satisfactory for the determination of chromosome numbers by the use of the acetocarmine smear method.

CHROMOSOME BEHAVIOR IN POLLEN MOTHER CELLS

The hybrids between maize and teosinte that were used in this study were F_1, F_2, back crosses, and more complicated crosses. The determination of the chromosome numbers of individual plants was

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[3] Unpublished manuscript.
[4] Reference is made by number (italic) to Literature Cited, p. 805.

most readily made at the first-division anaphase. Figure 1, *A–D*, shows this phase in an F_1, a back cross, a back cross again back-crossed to maize, and a self of a back cross, respectively.

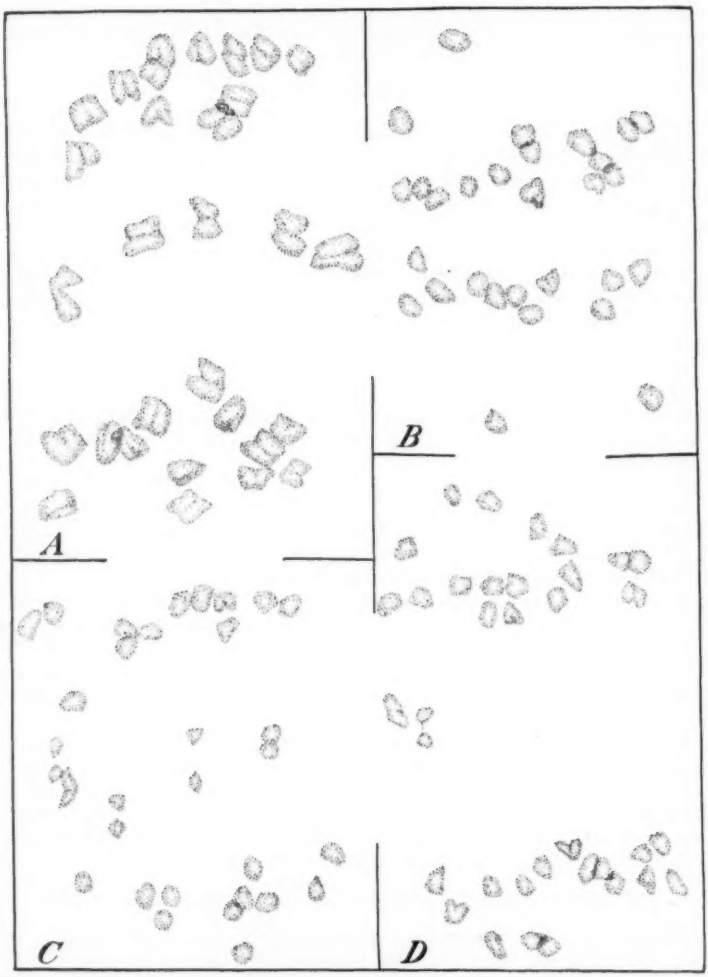

FIGURE 1.—First-division anaphases in teosinte-maize hybrids: *A*, 30 chromosomes of an F_1 plant; *B*, 29 chromosomes of a back cross; *C*, 28 chromosomes of a back cross crossed with maize; *D*, 35 chromosomes of a selfed back cross. *A* × 1,900; *B–D* × 1,200.

At this phase all bivalent chromosomes have divided and the univalents appear almost identical with the halves of the bivalents, so that a count of all chromosomes gives at once the $2n$ number of the plant under inspection. If the figure happens to be in late anaphase, a few of the univalents may be in the region of the plate in the process

of division. Even at this stage the character of the dividing univalents is such that they are not mistaken for bivalent chromosomes.

In 1924 the writer (*13*) reported the number and some of the characteristic features of the chromosomes in the developing pollen mother cells of F_1 plants of crosses between *Euchlaena perennis* and *Zea mays*. Since that time additional material has been available, and it has been found that with one exception, referred to later, all plants have 30 chromosomes, the sum of the haploid chromosome numbers of the two parents.

The behavior of the chromosomes in these hybrids during meioses of the pollen mother cells has been found to be that already described (*13*), except in a few minor details. A closer examination of the disposition of the univalent chromosomes, which are usually scattered on the spindle at the time the bivalent and trivalent chromosomes form the first-division metaphase plate, shows that many of them move undivided to the poles with the halves of the divided chromosomes. However, one or more of the univalents frequently will be found at the plate region when the divided chromosomes are in the anaphase, and at late anaphase these univalent chromosomes will divide. Except for the occasional undivided univalents or halves of univalents that are too far afield, the two daughter nuclei include the divided halves of the bivalent chromosomes and varying numbers of divided and undivided univalent chromosomes. Those chromosomes that fail to be included in the daughter nuclei are left in the cytoplasm to form micronuclei or to degenerate.

The second division is much more regular than the first. Only the halves of univalents present cause any appreciable abnormalities. Their position on the metaphase spindle is indefinite and their distribution is at random to the two poles.

The chromosome behavior during meiosis of the pollen mother cells of later hybrid generations is in most respects similar to that observed in F_1 hydrids. The chromosome numbers in F_2, in back crosses, and in others of the more complicated hybrids are likely to vary for each individual. The chromosome complement of such plants will be made up of chromosomes derived from the two parents. The number from either parent is unpredictable, and unfortunately cytological study has not yet developed any method of differentiating the chromosomes of maize from those of teosinte.

Camera-lucida drawings were made as a record of the chromosomes of each plant, since the temporary slides could not be preserved for any appreciable time. The drawings of first-division anaphases gave an opportunity to study the distribution of chromosomes to the two daughter nuclei resulting from the first meiotic division.

The distribution of the chromosomes in an F_1 hybrid seems generally to show that of the 3 sets of 10 chromosomes present 2 sets pair regularly and 10 chromosomes go to each pole, whereas the chromosomes of the third set are distributed at random to the two daughter nuclei. Figure 2, *A*, shows 10 bivalents, 1 pair of loosely associated chromosomes, and 8 univalents. In plants of later hybrid generations, however, the number of regularly pairing chromosomes may be more than 10, since a plant may have 4 or more homologous chromosomes instead of just 3 as in F_1 hybrids. Figure 2, *B* and *C*, shows cells having 22 chromosomes. In *B* all chromosomes are paired, and in *C* there are 10 pairs and 2 univalents, suggesting that in the former

there are 4 homologous chromosomes present, whereas in the latter the 2 unpaired chromosomes are not homologous.

The genetic data have shown that in F_1 plants and in back crosses on maize autosyndesis is the rule and allosyndesis the exception, so that in F_1 hybrids the unpaired chromosomes are from maize, whereas in plants from back crosses on maize the unpaired chromosomes are from teosinte.

Table 1 gives a comparison between the numbers of chromosomes that go to make up the daughter nuclei resulting from the first meiotic division of 30-chromosome hybrids and the numbers calculated when 10 paired chromosomes go at random to join the 10 divided bivalents at the two poles, i.e., the binomial expansion of $(a+b)^{10}$. Although the data of this table are made up from drawings of plants with different percentages of maize and teosinte chromosomes, it indicates that the number of chromosomes between 10 and 20 that enter a gamete depends upon chance. The binomial expansion does not represent the chance distribution of extra chromo-

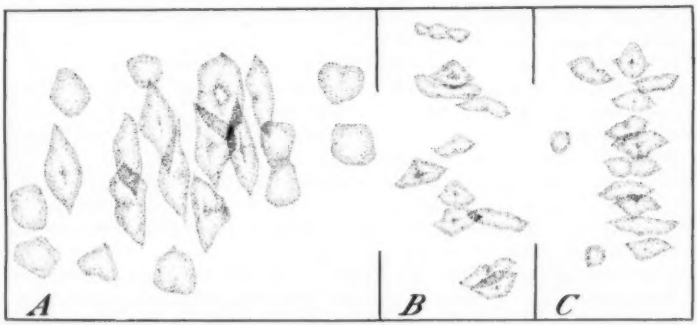

FIGURE 2.—First-division metaphase of teosinte-maize hybrids: *A*, 11 bivalent and 8 univalent chromosomes of an F_1 plant; *B*, 11 bivalent chromosomes of a later hybrid generation; *C*, 10 bivalent and 2 univalent chromosomes from a sister plant of *B*. *A* × 1,800; *B* and *C* × 1,200.

somes in any generation following F_1, for then the number of pairs is not limited to 10. For example, in a plant with 30 chromosomes it is possible to have 15 paired and no unpaired chromosomes, and in such a plant the distribution of the chromosomes at the first meiotic division invariably will be 15 chromosomes to each daughter cell. To calculate an expected chance distribution of chromosomes in 30-chromosome plants of perjugate generations it is necessary first to calculate the expected chance distribution of additional pairs and then apply a binomial distribution to the remaining unpaired chromosomes. Distributions have been calculated for plants with 22, 23, and 24 to 40 chromosomes, based on random distribution of the extra chromosomes from an unlimited number of sets of 10 chromosomes. The observed distributions of chromosomes in many of the plants with a few extra chromosomes are sufficiently close to the calculated distributions to verify the assumption that in later generations the distribution approaches this modified distribution more nearly than it does the simple binomial expansion of the number of chromosomes above 20 present in an individual. However, agreement with such a modified distribution of extra chromosomes does not conflict with

the statement that univalent chromosomes are distributed at random, but is simply a more exact method of estimating the number of unpaired chromosomes.

TABLE 1.—*Distribution of chromosomes to the 2 daughter nuclei resulting from the first meiotic division of 30-chromosome hybrids*

Chromosomes	Number of cells with indicated chromosome distribution					
	15–15	16–14	17–13	18–12	19–11	20–10
Observed	17	30	14	9	5	0
Calculated $(a+b)^{10}$	18.4	30.8	17.6	6.6	1.5	.00+

The data presented in table 1, supported by similar data from drawings of chromosomes of hybrids having chromosome numbers

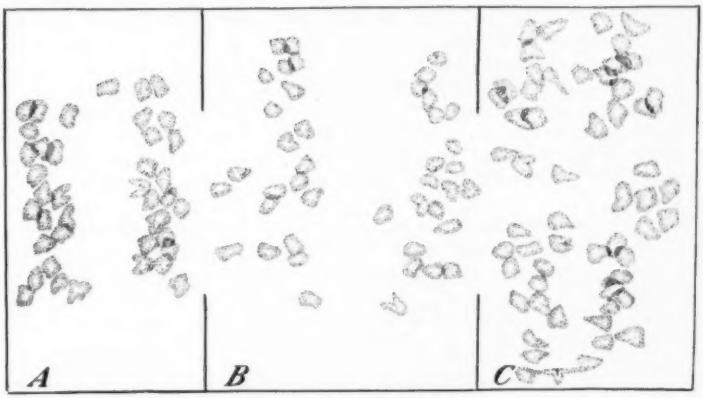

FIGURE 3.—First-division anaphases of teosinte-maize hybrids with aberrant chromosome numbers: *A*, 40 chromosomes from an F_1 plant; *B*, 42 chromosomes from a plant of a progeny twice back-crossed on maize; *C*, 56 chromosomes from an F_2 plant. × 1200

other than 30, indicate that there is a random distribution of all unpaired chromosomes and that gametes are produced with all chromosome numbers in approximately the predicted numbers in each group, the number of chromosome groups depending upon the number of univalent chromosomes present to be distributed at random.

ABERRANT HYBRID PLANTS

In a progeny of 7 F_1 hybrids grown in 1932, it was noticed that 1 plant was markedly different from the 6 sister plants or from any of the previously grown F_1 hybrids. The ears were generally 4-rowed, the pollen showed very little sterility, and abundant seeds were produced after either cross-pollination or selfing. This plant was found to have 40 chromosomes (fig. 3, *A*). The fact that it seemed more cornlike than other F_1 plants suggested that this increase of 10 chromosomes above that of normal F_1 plants was due to the presence of two sets of corn chromosomes. Perhaps the most logical

assumption to account for the double number of chromosomes is that doubling took place soon after fertilization; but other possibilities, such as double fertilization, make it unwise to speculate.

Emerson and Beadle (*6*) have described a similar aberrant F_1 plant, and the present plant agrees very closely with their description. Such plants, as they suggest, may offer possibilities for genetic and cytological studies.

A small F_2 population from the aberrant plant was grown. The plants were very uniform in appearance, and chromosome counts showed that all of them had 40 or approximately 40 chromosomes.

A second aberrant plant appeared in a progeny twice back-crossed with maize. Sister plants of this progeny were found to have chromosome numbers ranging from 20 to 28, whereas this plant had 42 chromosomes (fig. 3, *B*). Such a high number suggests a doubling of all chromosomes after fertilization. Although all the experimental plants resulted from guarded pollination, outcrossing might explain the high number of chromosomes; but such an explanation is unlikely, since the plant had 14 more chromosomes than were found in the sister plant having the next highest number. To produce a plant with 42 chromosomes would require a functioning pollen grain with 24 or more chromosomes, a number exceptionally high for the pollen of any hybrid that has yet been grown. The presence of the *wx* gene further reduced the probability that the plant resulted from outcrossing, since no plants were being grown that could produce pollen grains having high numbers of chromosomes and the *wx* gene.

Two plants, one with 56 chromosomes (fig. 3, *C*), from the F_2 progenies of teosinte and maize, have had high chromosome numbers that suggested a doubling of the chromosomes contributed by either parent or by both. The occurrence of these plants with high chromosome numbers is in keeping with the finding of similar abnormal hybrid plants by investigators of other plant groups.

CHROMOSOME MORPHOLOGY

The investigations of McClintock (*16*) and Beadle (*1*) have stimulated an attempt to search the midprophase of some of the hybrid plants, since in many forms there are three allelomorphic chromosomes and often it would be very useful if such chromosomes could be identified. A few plants having only 21 chromosomes are known to be trisomic for chromosome no. 9, the chromosome that carries the *wx* gene, and it seemed that such plants would be useful for a preliminary study. This study, however, has proved disappointing, partly because of the lack of any definite morphological knowledge of the chromosome complement of *Euchlaena perennis*. It has not been difficult to identify chromosome no. 5 (of *Zea*) by its characteristic attachment to the nucleolus. A short chromosome with a terminal knob on the short arm was quite frequently lying where it could be traced and drawn. This chromosome resembles McClintock's drawings of chromosome no. 9, but Beadle (*1*) has shown that terminal knobs are prevalent on the chromosomes of Florida teosinte. It seems unsafe, therefore, to ascribe a definite identification to such a chromosome in these hybrid plants.

TABLE 2.—*Chromosomes in back-crossed and F_2 Euchlaena perennis–Zea mays hybrids*

Female parent		Male parent		Number of plants with indicated chromosome number																								Mean chromosome number		
Species or cross	Chromosomes	Species or cross	Chromosomes	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	Total plants	Observed	Expected	Of functioning gametes of hybrid plant
	No.		No.																								No.			
E. perennis × *Z. mays*	30	*Z. mays*	20	1	1	1	1	5	5	5	2	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	26	25.3	25	15.3
Z. mays	20	*E. perennis* × *Z. mays*	30	...	...	...	...	1	1	1	4	24	66	8	3	5	14	23	29	31	19	5	5	...	...	1	102	29.8	25	19.8
E. perennis × *Z. mays*	30	do	30	...	...	...	...	...	...	...	...	...	1	1	1	...	...	...	...	...	...	...	...	...	...	...	140	35.94	30	...

A disconcerting observation of the threads of midprophases was the absence of univalent threads in plants known to have one or often several univalent chromosomes. Beadle (*1*) has referred to a similar absence of univalent threads in hybrids of Florida teosinte and maize. A further study of what seems to be very suitable material is being made in an attempt to definitely trace the behavior of the univalent chromosomes in early prophase stages.

CHROMOSOME SELECTION IN GAMETES

The study of the chromosome numbers prevalent in the functioning pollen and ovules of any particular teosinte-maize hybrid is most readily determined by making reciprocal crosses on maize in which the gametes are known to have 10 chromosomes. By subtracting 10 chromosomes from the chromosome numbers of the plants of the back-crossed progeny, the chromosome number of the gametes of the hybrid parent is obtained. The data of tables 2, 3, and 4 summarize the observed chromosome numbers of the plants of these and other more complicated progenies.

The data of table 2 give the chromosome counts of 26 back-crossed plants in which the F_1 was the female parent, of 102 in which the F_1 was the male parent, and of 140 F_2 plants.

The mean chromosome number of the 26 plants used to determine the chromosome number of the functioning ovules of F_1 plants is 25.3. If 10 is subtracted, the mean number for functioning ovules becomes 15.3, which is just slightly above the expected mean for a 30-chromosome plant. The distribution of these plants among chromosome groups is approximately random.

The mean chromosome number of the 102 plants used to determine the chromosome number of the functioning pollen of F_1 plants is 29.8. If 10 is subtracted, the mean number for functioning pollen becomes 19.7, a number far above the expected mean. The distribution is also very limited, with a range of but 4 chromosomes.

Three plants produced by using F_1 pollen on *Euchlaena perennis* have a mean chromosome number of 39.66, thus showing that the gametes of the F_1 that functioned in their production had 19.66 chromosomes, a number that agrees very closely with 19.8 obtained from the larger population.

It is apparent that the chromosome number of the functioning ovules is in agreement with the chromosome numbers observed going to form the daughter nuclei at the first meiotic divisions. In the pollen, however, there is a marked discrepancy between the observed chromosome distribution at the time the pollen is formed and the chromosome numbers found in functioning pollen. The mean chromosome number, 19.8, shows clearly that there is a tendency for functioning pollen to be restricted to gametes with 20 chromosomes.

In the F_2 progeny the chromosome number ranges from 25 to 42, with a mean number of about 36. This is approximately the mean number expected from combining the mean numbers for functioning ovules and pollen. The distribution approaches that expected from combining the distribution of the ovules and pollen. Three plants, however, with the chromosome numbers 25, 26, and 27, respectively, seem to be sufficiently segregated from the major group to suggest that these three plants were produced from pollen with approximately 10 instead of 20 chromosomes.

TABLE 3.—*Chromosomes in back crosses on Zea maya and selfed back crosses*

Female parent		Male parent		Number of plants with indicated chromosome number																						Total plants	Mean chromosome number		
Species or back cross	Chromosomes	Species or back cross	Chromosomes	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40		Observed	Expected	Of functioning gametes of hybrid parent
	No.		No.																						No.			
Z. maya × (*E. perennis* × *Z. maya*)	30	*Z. maya*	20	8	13	20	32	25	16	10	4	1	2	....	1	....	....	....	....	....	....	....	....	....	132	23.44	25	13.44
Z. maya	20	*Z. maya* × (*E. perennis* × *Z. maya*)	30	6	1	1	....	....	1	2	3	14	13	5	1	....	....	....	....	....	....	....	....	....	47	27.87	25	17.87
Z. maya × (*E. perennis* × *Z. maya*)	30	do	30	4	1	5	3	3	4	2	5	1	5	12	6	14	22	28	25	17	8	10	....	4	179	32.44	30	....

Table 3 presents the data from back-crossed plants that have been back-crossed again onto maize or selfed. The hybrid parents given in this table have 30 chromosomes, and so do not differ in chromosome number from the F_1 plants given in table 2. The chromosome complement has been appreciably changed, however, for instead of the 30 chromosomes being 20 teosinte and 10 maize, as is found in F_1 plants, there will be approximately 10 teosinte and 20 maize chromosomes if autosyndesis has been general.

The mean chromosome number of the functioning ovules of these back-crossed plants (having two sets of maize chromosomes in their chromosome complement), as measured by the chromosome number in plants from back crosses crossed with maize, is 13.44. This mean number is appreciably less than that found for the functioning ovules of F_1 plants and suggests that there is a tendency in this group of hybrids with a predominance of maize chromosomes to develop those ovules with chromosome numbers approximating the haploid chromosome number of maize.

The mean chromosome number of the functioning pollen of 30-chromosome back-crossed plants is 17.87. This number is also appreciably less than that found for F_1 plants. The more striking difference, however, is the bimodal distribution shown by these 47 plants, a distribution that was absent in the 102 plants used to measure the chromosome number of F_1 pollen, although suggested in the discussion of the slight bimodal tendency observed in the chromosome number of F_2 plants. The grouping of the chromosome numbers with one mode at 20.25 and another at 29.2 seems to demonstrate that functioning pollen tends to approach very closely the haploid chromosome numbers of maize or teosinte.

The mean chromosome number of selfed 30-chromosome back-crossed plants is 32.44 which is a little above the sum of the means of the chromosome numbers of the functioning ovules and pollen of back-crossed plants. The difference, however, is not significant, and the distribution of the plants among the various chromosome classes, although too complicated for a complete analysis, suggests two modes—one a little below 25 and the other a little below 35.

Table 4 is made up of additional data for various hybrids. It is apparent that in those progenies in which the female ancestor is the hybrid parent and the male is maize the chromosome numbers of functioning ovules of the female ancestor tend to approach the basic number, 10; but this tendency, although apparent, is sufficiently flexible to allow ovules with any chromosome number between 10 and 20 to function. Some additional data are presented to show the chromosome numbers of pollen of several complicated hybrids. The male ancestors of these progenies, largely maize in their composition, are found to have, in functioning pollen, chromosome numbers approximating the basic number, 10.

In no plant used to show the chromosome numbers of functioning pollen has a gamete been found having 23, 24, or 25 chromosomes—numbers that in many progenies would be expected to predominate. This is a clear indication that the chromosome numbers in functioning pollen are modifications of the numbers that are prevalent in the gametes at the time of their formation. Such modifications apparently result from a differential death rate during development or from a differential growth rate down the pollen tubes, or from both.

TABLE 4.—*Chromosomes in various Euchlaena perennis-Zea mays hybrids*

Female parent		Male parent		Number of plants with indicated chromosome number																		Plant	Mean chromosome number		
Species or cross	Chromosomes	Species or cross	Chromosomes	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38		Actual	Expected	Of functioning gametes of hybrid parent
	No.		*No.*																				*No.*			
Z. mays × (*E. perennis* × *Z. mays*)	20	*Z. mays*	20	1	---	3	2	---	1	---	---	1	---	---	---	---	---	---	---	---	---	---	8	23.12	24.5	13.12
Z. mays	20	*Z. mays* × (*E. perennis* × *Z. mays*)	31	6	1	1	---	---	---	---	1	1	10	2	---	---	---	---	---	---	---	---	22	25.82	25.5	15.82
Z. mays × (*E. perennis* × *Z. mays*)	28	do	28	---	1	1	---	---	---	---	---	---	---	---	2	4	2	---	---	---	---	---	10	29.90	28	---------
Do	29	do	29	---	1	4	3	6	1	2	---	---	---	---	1	1	2	3	2	---	---	---	26	26.92	29	---------
Do	31	do	31	---	1	1	---	1	---	---	---	1	---	1	1	1	1	3	2	4	2	---	19	32.16	31	---------
Z. mays × (*E. perennis* × *Z. mays*) S. P.[a]	27	*Z. mays*	20	5	7	7	9	3	2	2	---	---	---	---	---	---	---	---	---	---	---	---	35	22.34	23.5	12.34
Do	33	do	20	---	---	3	11	22	29	26	5	2	1	---	---	---	---	---	---	---	---	---	99	24.93	26.5	14.93
Z. mays	20	*Z. mays* × (*E. perennis* × *Z. mays*) S. P.[a]	27	70	14	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	85	20.19	23.5	10.19
(*Z. mays* × (*E. perennis* × *Z. mays*) × *Z. mays*.	22	*Z. mays*	20	25	25	10	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	60	20.75	21	10.75
Z. mays	20	(*Z. mays* × (*E. perennis* × *Z. mays*)) × *Z. mays*.	22	60	1	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	61	20.02	21	10.02
Z. mays × (*Z. mays* × (*E. perennis* × *Z. mays*)).	29	*Z. mays* × (*Z. mays* × (*E. perennis* × *Z. mays*)).	29	1	2	4	3	2	2	2	---	---	---	---	---	1	---	1	---	---	---	---	18	24.17	29	---------
Do	31	do	31	---	---	---	---	1	1	---	---	---	---	2	1	2	3	1	2	2	1	1	17	32.59	31	---------
Z. mays (F_2)	29	*Z. mays*	20	---	4	17	10	14	12	5	1	---	---	---	---	---	---	---	---	---	---	---	63	23.51	24.5	13.51
Do	31	do	20	---	---	6	8	---	7	2	1	---	---	---	---	---	---	---	---	---	---	---	24	23.75	25.5	13.75
Z. mays	20	*Z. mays* (F_2)	29	22	3	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	25	20.12	24.5	10.12

[a] Selfed progeny.

The data presented in table 4 to show the chromosome numbers prevalent in selfed progenies do not seem to merit extensive analysis, but they do indicate a bimodal tendency that must be due in large measure to the differential functioning of pollen having the basic chromosome numbers of either the maize or the teosinte parents.

DISCUSSION

The foregoing data indicate that in hybrids between *Euchlaena perennis* and *Zea mays* pollen effective in fertilization has a chromosome number approaching the chromosome number of the pollen of either of the ancestral forms, i.e., 10 or 20. In F_1 plants, as measured in back crosses, there is a very marked tendency for pollen with 20 chromosomes to effect fertilization. However, three F_2 plants with low chromosome numbers suggest that pollen with about 10 chromosomes has occasionally functioned.

That pollen grains with 10 chromosomes do, in fact, occasionally function is substantiated by the chromosome counts made in back crosses of various hybrids on corn. The functioning pollen from most back crosses on corn has approximately 20 chromosomes, but one progeny included in the data showed that pollen effective in fertilization approached the two extremes, 10 and 20, in about equal numbers. The pollen from the more cornlike hybrids derived from crossing a back cross onto corn had invariably 10 or approximately 10 chromosomes.

All studies made of many different types of hybrids between teosinte and maize have shown a complete absence of pollen with 13, 14, and 15 chromosomes. The high percentage of empty pollen grains characteristic of the F_1 plants indicates that there has been an elimination of pollen through death early in its development. In other hybrids, however, there is conclusive proof that after pollination there is a differential death or growth rate which eliminates pollen other than that having approximately 10 and 20 chromosomes. This is shown by contrasting the percentage of waxy pollen in mature pollen and the percentage of waxy seeds obtained when this pollen is used to pollinate homozygous waxy plants.

The data accumulated to determine the number of chromosomes in functioning ovules tend to show that ovules are distributed at random among the various classes. The mean chromosome number observed is frequently not a significant departure from the calculated mean. There are, however, in some of the more cornlike progenies significant departures that show a tendency for the selection of gametes with low chromosome numbers. It seems, therefore, that in the ovules there is a slight tendency to favor the survival of the parental chromosome numbers, but the data are too meager to show a bimodal distribution even if it was present. It is evident, however, that the differential survival of gametes possessing parental numbers is much less marked in the ovules than in the pollen.

The effect of the pronounced tendency for euploid gametes to be more virile—a tendency very pronounced in the pollen and suggested in the ovules of teosinte-maize hybrids—is to produce many plants with 20 and 40 chromosomes.

The chromosome complement, however, in these 20- or 40-chromosome plants is profoundly affected by the prevalence of autosyndesis. A hybrid with 2 sets of teosinte chromosomes will produce gametes with 1 set of teosinte chromosomes irrespective of the number

of maize chromosomes present, or if the 2 sets of chromosomes are from maize there will be 1 set of maize chromosomes in all gametes formed. Such a chromosome distribution leads to the presence of 2 sets of teosinte chromosomes in all F_2 plants and 2 sets of maize chromosomes in all plants of selfed progenies from back crosses on maize.

The number of maize chromosomes in F_2 progenies and in back crosses on maize and the number of teosinte chromosomes in selfed progenies of these back crosses depend upon the absence of complete autosyndesis and upon the utilization of gametes with aneuploid or with the diploid chromosome numbers.

Cytologists have frequently reported that in hybrids between parents differing in chromosome number there occurs a differential survival of gametes bearing the parental number of chromosomes.

Täckholm (*21*), in his study of polyploid roses, showed that in the *canina* roses there is a marked tendency to mature pollen with a chromosome number near the basic number, 7. He has shown, on the other hand, that this class of roses makes ovules with only 7 chromosomes less than the somatic number of the plant under investigation. Blackburn and Harrison (*2*) have shown that in a pentaploid rose the functioning pollen has 7 chromosomes and that the egg cells have 28.

The elimination of all pollen except that with 7 chromosomes is in harmony with the selection found in many progenies of *Euchlaena-Zea* hybrids in which only those pollen grains functioned that had the basic chromosome number 10 or approximately 10.

The studies of the distribution of chromosomes in wheat hybrids and the number of chromosomes in functioning gametes is touched upon by Sax (*18*). In a later paper (*19*) he goes more fully into the chromosome number of functioning gametes of a 35-chromosome hybrid and finds that both male and female gametes have 14 chromosomes more frequently than would be expected on the assumption of a random distribution of 7 single chromosomes. Thompson (*22*), and more recently Thompson and Cameron (*23*), find that in a similar wheat cross there is an elimination of gametes with chromosome numbers between the two modes 14 and 21, which is more pronounced in the pollen but significant in the ovules. Watkins (*24*) finds that in a pentaploid wheat hybrid the ovules are generally fertile, whereas in the pollen grains there is a high degree of sterility. He finds further that pollen grains possessing either 14 or 21 chromosomes are more likely to function than those having other numbers of chromosomes.

The random distribution of unpaired chromosomes of wheat hybrids at the time the gametes are formed and the later elimination of gametes with chromosome numbers between the two extremes, as described by the foregoing investigators, are very similar to the conditions found in this study of *Euchlaena-Zea* hybrids. In these hybrids gametes with all chromosome numbers between the two modes are formed, but in the pollen and to a lesser degree in the ovules there is a marked tendency to eliminate all but the low and the high chromosome numbers.

The studies by Goodspeed, Clausen, and Chipman (*10*) and by Goodspeed and Clausen (*9*) of *Nicotiana* hybrids show in some cases that a triploid distributes its 12 univalent chromosomes at random in

the first division and that there is a random survival of pollen in the different chromosome classes at the time of fertilization.

Karpechenko (*12*) has found that viable gametes of F_1 hybrids of *Raphanus* × *Brassica* ($2n = 18$ chromosomes), usually have 18 chromosomes—9 *Raphanus* and 9 *Brassica*. The pollen of F_1 *Euchlaena-Zea* hybrids is similar in constitution to that described by Karpechenko, since in both cases there is reason to believe that the chromosome complement of most of the functioning pollen is made up of one set from each of the parents.

Blakeslee and Farnham (*3*) found that daturas having $2n+1$ chromosomes produce ovules with n, $n+1$, and $n+2$ chromosomes, whereas practically no pollen that functions carries extra chromosomes. McClintock (*15*) has shown that in *Zea* plants which have extra chromosomes there is a decided selection against extra chromosomes in the male gametes and a less obvious selection against eggs carrying extra chromosomes.

It is apparent that selection of gametes with chromosome numbers approaching the basic number or a multiple of the basic number present in the hybrid's ancestors has occurred in widely separated groups of plants. In some cases the gametes have borne the haploid number of chromosomes and in others the diploid number. In most cases it seems that the gametes formed represent all chromosome numbers between the two extremes and that the selection takes place either by differential viability or by differential growth rate. One case, namely, that of the roses, has been described where the formation of ovules is irregular and the eggs lack only 7 chromosomes of the somatic chromosome number characteristic of the hybrid.

Where the chromosome complement of a plant is made up of chromosomes in addition to the two homologous sets, the tendency of the functioning gametes to have the basic chromosome number or a multiple of this number must lead to the production of plants with chromosome numbers in multiples of the basic number and to the absence of plants with aneuploid chromosome numbers.

Indirect demonstration of the selection of gametes is seen in the chromosome numbers of a plant group such as *Rubus* (*8*) or other similar groups which are found to have chromosome numbers only in multiples of the basic number and in which hybridization frequently occurs between forms with different chromosome numbers. The lack of aneuploid forms in such groups seems to be the result of gametic selection favoring the parental numbers.

If triploid hybrids utilized only euploid gametes, three groups of plants, namely, diploid, triploid, and tetraploid, would be found in the offspring.

The tetraploid plants represent a fairly stable group distinct from either parent and contain two sets of chromosomes of the two parents if autosyndesis has been the rule. Darlington (*5*) has stressed the value of such hybrids in productive plant breeding. It is true that in tetraploids there is a proportionate increase in genes with an increase in chromosomes, but a crypt hybrid may breed true for many generations with only rare indications of its hybrid nature. If autosyndesis prevails, a tetraploid may defy our best efforts to segregate and stabilize hidden genetic characters.

Gigantism has frequently been associated with marked increase in chromosome numbers, and consequently tetraploids should be desir-

able in cases where gigantism is a desired factor. Tetraploid perennial teosinte, however, is no larger than the diploid annual form.

Gregory (*11*) and Sinotô (*20*) have demonstrated that gigantism occurs in forms having few chromosomes as well as in forms having many. Frost (*7*) and Longley (*14*) have found that tetraploid citrus plants are dwarfs as compared with their diploid relatives.

It seems to have been rather generally accepted that in isolating improved strains from polyploid hybrids the progenies with a large number of chromosomes provide the most desirable material. The idea may have originated from the cases of gigantism associated with a doubling of the chromosome number or from the fact that improved varieties tend to have a higher chromosome number than unimproved or wild stocks.

It has been pointed out that only a small percentage of tetraploid forms exhibit gigantism. The two tetraploids produced in the present study showed no increased vigor. The increased number of chromosomes in improved varieties may be accounted for in another way. Chromosome number is a character which was not considered in the breeding of existing varieties but which has been observed since the varieties were developed. If favorable variations occurred in the same ratio in diploid and tetraploid derivatives but if tetraploid derivatives were more numerous, high chromosome numbers would predominate in the varieties finally produced. In the descendants of the maize-perennial teosinte hybrids the mean chromosome number is well above the mean of the parents, but no correlation between chromosome number and vigor could be detected.

The final question to consider is that of the bearing of chromosome number on the recombination of parental characters and the ease with which desired combinations can be stabilized.

In triploid hybrids where only euploid gametes function, the isolation and stabilization of a new combination of genes is possible only when allosyndesis occurs in the pairing of the homologous chromosomes. If autosyndesis always occurs, the tetraploid segregates will combine the parental characters in heterozygous forms and these forms will reproduce themselves as long as autosyndesis continues to prevail, but no new homozygous combination of genes is possible. When allosyndesis occurs, three groups of segregates, namely, diploid, triploid, and tetraploid, are obtained, all of which combine the ancestral characters, and from any form containing the desired combination homozygous plants can eventually be isolated.

Because of the formation of both haploid and diploid gametes, the triploid segregates are too complex to be discussed here, but the relative advantage of high and low chromosome numbers may be estimated from diploid and tetraploid derivatives. Assuming that the triploid hybrid is heterozygous for two simple characters located in different chromosomes and that is is desired to fix a nonparental combination of the characters, it follows that:

(1) In the haploid gametes the four possible combinations of dominants and recessives will be represented in equal numbers, and in selfed F_2 individuals 1 out of 16 will be homozygous for any desired combination.

(2) In the diploid gametes there will also be four classes, but none of them will be pure for nonparental combinations. Three quarters or nine sixteenths of the selfed F_2 individuals will carry the desired com-

bination but they will not be homozygous; it will therefore be necessary to grow a third or even a fourth generation before the combination can be obtained in a homozygous form.

Consequently it is apparent that a diploid segregate supplies all the parental combinations that may be found in a tetraploid segregate and offers a distinct advantage when an attempt is made to isolate and stabilize any particular combination.

The behavior of F_1 teosinte-maize hybrids has shown that it is possible to obtain from a tetraploid form a diploid form having a chromosome complement made up of 2 of the 4 sets of the tetraploid. From selfing back crosses on maize, plants may be obtained having chromosome complements made up entirely from the chromosomes of the diploid parent.

These findings suggest the possibility that diploid maize may have originated from a hybrid between tetraploid perennial teosinte and some unknown diploid relative. It is much simpler, however, to assume that this hybridization occurred between two diploid forms whose blended characters produced the ancestor to maize, and that *Euchlaena perennis* perhaps does not represent a remnant of a once-prevalent form but rather a recent tetraploid form of the widely distributed annual teosinte.

In the foregoing general discussion of gametic selection in hybrids whose ancestors differed in chromosome number, as well as in the particular discussion of gametic selection in *Euchlaena-Zea* hybrids, the point is emphasized that the selective survival of gametes will profoundly affect the nature of the forms recovered in later generations. The illustrations are not perfect examples of the survival of gametes possessing the basic chromosome number (or multiples of this number) of the form involved. If this tendency were perfect (and it does not seem unreasonable to assume that in many cases it is) its effect on future offspring would be apparent. It seems probable that in nature this differential survival has produced as many recombinations with low as with high chromosome numbers. In this connection the occasional appearance of tetraploids must be cardinal, forming part of an evolutionary process that tends to keep the chromosome number in plant groups of recent origin no higher than those found in the older groups.

SUMMARY

The meiotic behavior of the chromosomes in various teosinte-maize hybrids indicates that homologous chromosomes usually pair and that the unpaired univalent chromosomes are distributed at random to the daughter nuclei. The division of these univalent chromosomes occurs in either the first or the second division.

The regular distribution of all paired chromosomes and the random distribution of all unpaired chromosomes result in gametes with various chromosome numbers ranging from the number of pairs to the number of pairs plus the number of unpaired chromosomes.

The chromosome number of functioning pollen of the various teosinte-maize hybrids is found to approximate either 10 or 20, the haploid chromosome numbers of the two parents, while the chromosome number of functioning ovules shows only a slight tendency toward the euploid numbers, 10 and 20.

An increased viability of euploid gametes seems to be general in hybrids similar to the teosinte-maize hybrids here described, and the survival of gametes with these chromosome numbers profoundly affects the character of the progeny of hybrids.

It is suggested that the diploid derivatives of hybrids offer favorable experimental material. If allosyndesis has occurred, even the gametes with the haploid chromsome number of the diploid parent will contain chromosomes and consequently characters from both parents. A diploid derivative will, therefore, combine the ancestral characters of the parents in a plant that seems just as promising for breeding material as those forms with twice as many chromosomes.

The utilization of euploid gametes from tetraploid hybrids restricts the plants of their progenies to three chromosome groups; namely, diploid, triploid, and tetraploid. The prevalence of diploid forms combining the characters of the ancestors suggests that gametic selection has led to the production of new and eventually stable forms without increasing the chromosome number above that of the diploid parent.

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A BACTERIAL DISEASE OF HEDERA HELIX [1]

By RICHARD P. WHITE, *research specialist, diseases of ornamentals, Department of Plant Pathology, New Jersey Agricultural Experiment Station,* and LUCIA MCCULLOCH, *associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

In the spring of 1930 a shipment of English ivy, *Hedera helix* L., was received in New Jersey from Maryland, heavily infected with a bacterial leaf-spot disease. These plants were grown outdoors during the summer, and infections failed to appear on the new growth. Several thousand cuttings were taken from them. In the fall, when these rooted cuttings were potted and grown under greenhouse conditions, the disease reappeared in epidemic form, killing many plants and rendering the remainder worthless. Since a bacterial leaf-spot disease of this host had not previously been mentioned as occurring in the United States, and since under certain conditions it causes serious losses, investigations of its cause and nature were undertaken. The results are reported in this paper.

LITERATURE REVIEW

Lindau (*5*) [2] in 1894 described a bacterial leaf-spot and stem-canker disease of English ivy in Germany. From his description and illustrations there is no doubt as to the identity of our material and his. No inoculation tests were attempted by Lindau. In his cytological work he failed to find evidence of stomatal infection and concluded that infection took place through the natural wounds on the stem caused by the sloughing off of the pubescence normally present on the tips of young growing stems. Rapidly growing plants were noted as being more susceptible than slower growing plants.

Arnaud (*1*) in 1920 redescribed the disease from France, giving the name *Bacterium hederae* to the organism he isolated. Although he failed to describe the organism or to report any inoculation trials to prove its pathogenicity, his description of the symptoms again leaves little doubt as to the identity of the disease.

Killian (*3*) in 1921 reported successful inoculations with *Bacterium hederae* Arnaud, thus proving the pathogenicity of the associated organism. He also described gross characteristics of the organism on several media. The incubation period was determined as from 1 to 3 weeks, depending on the temperature and humidity. He failed to obtain infection on old plant parts, even where wounded.

The first mention of this disease in the United States was made by White [3] in December 1930, followed by rather complete descriptions in August 1931 (*8*).[4]

[1] Received for publication Dec. 1, 1933; issued July 1934. Cooperative investigations between the New Jersey Agricultural Experiment Station and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture. Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Plant Pathology.

[2] Reference is made by number (italic) to Literature Cited, p. 815.

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Burkholder and Guterman (*2*) have recently described the same trouble on plants shipped from Georgia to New York and have reported synergism as existing between *Bacterium hederae* and an associated organism also isolated from diseased areas.

ECONOMIC IMPORTANCE

The ivy disease described here has been reported from two commercial nurseries in New York (*2*) and New Jersey, respectively, into which it was imported on plants purchased from growers located in Georgia and Maryland. In 1933 it was found on outdoor-grown ivy in Virginia and in the District of Columbia. Its geographic distribution and importance in the Southern States has not been investigated. Burkholder and Guterman (*2*) give no data on its seriousness in New York.

In New Jersey it immediately became a serious pest in the one large commercial greenhouse into which it was introduced in 1930. Cuttings taken from the stock plants were seriously infected. Over 40,000 were either killed outright or so severely infected that they were discarded as useless. Infections on plants trained into pyramids rendered them unsalable. Under greenhouse conditions where ivy is syringed periodically the spread of the disease is rapid, owing to the dissemination of the bacteria from infected leaf areas and stem cankers by the water. Introduced on *Hedera helix*, this disease has spread to several of its horticultural varieties being grown by this same concern and has persisted and caused injury and losses in spite of all efforts to check it.

SYMPTOMS

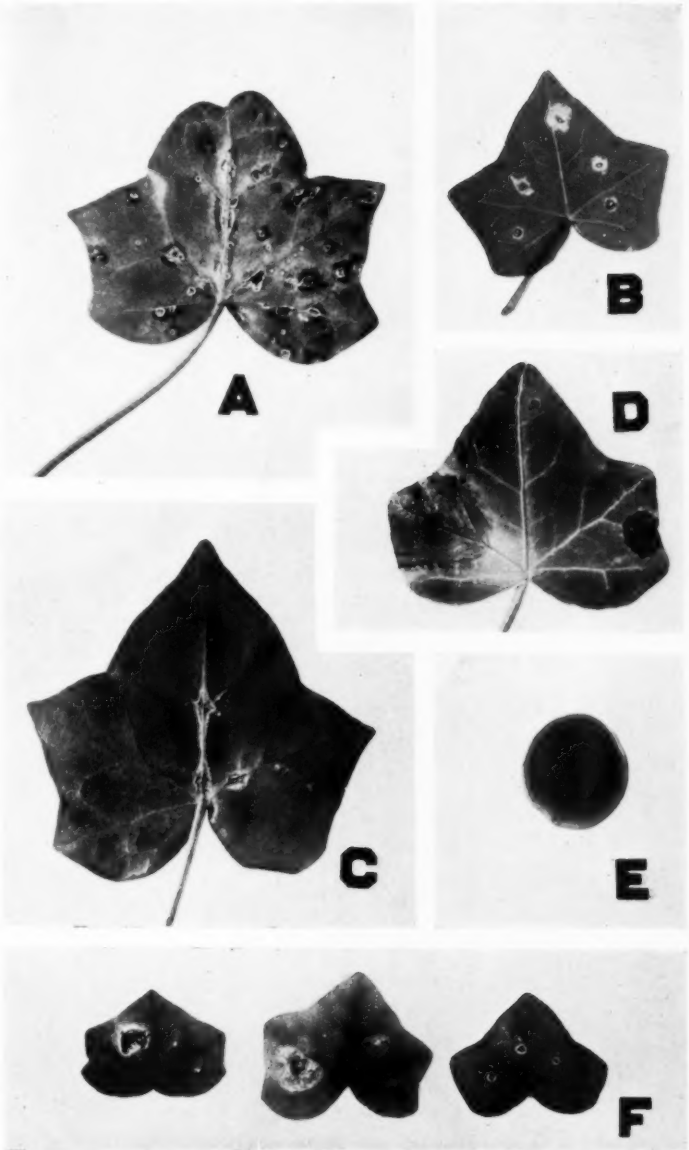
ON LEAVES

Recent infections on young leaves become evident in from 5 to 12 days as small translucent, roughly circular spots (pl. 1, *B*). In the earliest stages these spots are very difficult to see except by transmitted light. As the spots enlarge, the center becomes brown to brownish black, dries out, and frequently cracks (pls. 1, *A*, and 2, *A*). Under conditions of high humidity an orange-red bacterial exudate may occur on the infected areas. The older spots are usually surrounded by a light yellowish-green water-soaked area, but on old foliage this may be replaced by a reddish to reddish-brown irregular or scalloped region. Infected areas on the leaves are frequently secondarily infected with either *Colletotrichum trichellum* (Fr.) Duke (pl. 1, *D*) or *Phyllosticta hedericola* Dur. and Mont. This succession of *P. hedericola* following bacterial infections has previously been reported by Nicolas and Aggery (*6*) on *Aralia japonica*.

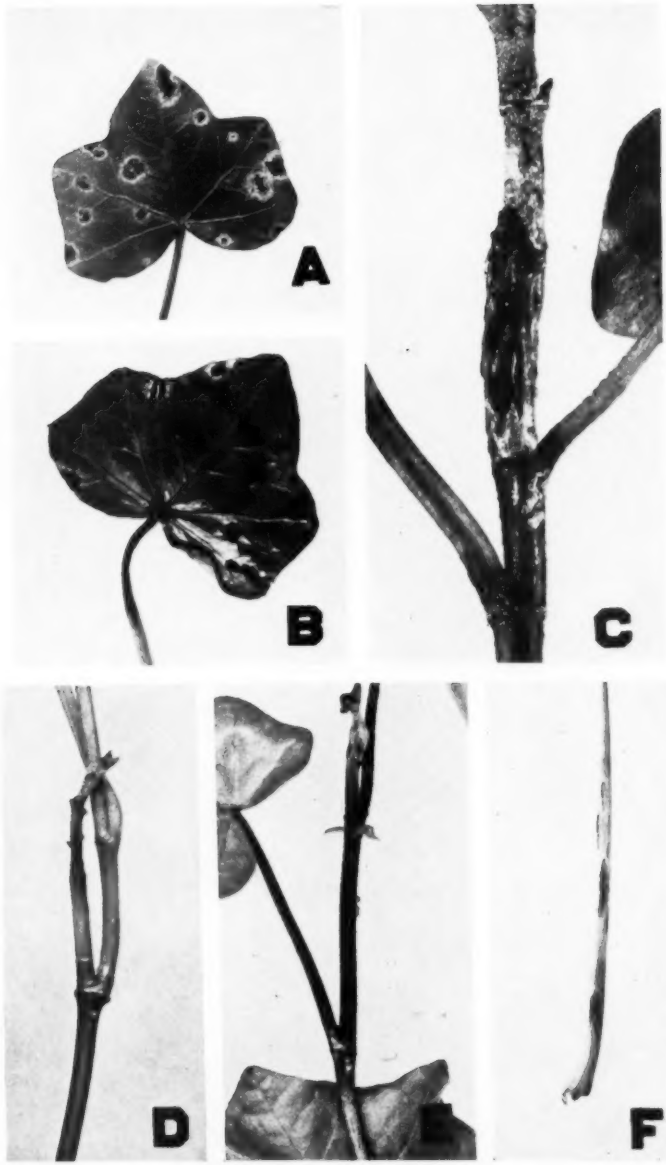
Infection frequently takes place on or very close to a vein. Under such conditions the spots developing are not circular but are elongated in the direction of the vein, indicating possible systematic invasion (pl. 1, *C*). Heavily infected leaves usually turn yellow and fall.

ON PETIOLES

Direct infection of petioles is rare. The spots that develop from such infections are dark brown to black and enlarge rapidly in both directions and soon girdle the petiole, and the attached leaf wilts (pl. 2, *B*, *F*). Petioles frequently become infected from heavily



A, Artificial inoculations obtained by brushing bacterial suspension on leaf with camel's-hair brush; B, needle-prick inoculations on young leaf after 8 days; C, artificial inoculations obtained by needle pricks in veins; D, leaf with four natural infections with *Bacterium hederae*, one of which has been followed by *Colletotrichum trichellum;* E, colony of *Bact. hederae* on beef-extract agar; F, leaf on left inoculated with *Bact. hederae;* center, double inoculation with *Bact. hederae* and culture *d;* right, double inoculation with *Bact. hederae* and culture *k;* all 4 weeks after inoculation.



A, Natural leaf infections; B, natural leaf infections and petiole lesions; C, stem canker; D, infection at growing tip; E, bacterial exudate from very young stem canker; F, petiole lesions inoculated by bacterial suspensions brushed on petiole with camel's-hair brush.

infected leaves, however, the bacteria advancing rapidly down the petioles to the stem.

ON STEMS

Infection on stems takes place naturally either from infected petioles or on the very young and tender growing tip (pl. 2, *D*). On young tissues a soft dark-brown to black decay rapidly takes place. Invasion is retarded when older tissue is reached, a new growing point is developed from the next lowest axillary bud, and no further advance is made.

On older tissues of the stem, where infection arises from petioles, definite cankers are produced. At first these cankers appear as small brown sunken areas. Invasion of tissue is slow. Old cankers are flattened and shrunken, usually cracked longitudinally, and surrounded by swollen margins due to callus formation on the part of the host (pl. 2, *C*). Stem cankers on old tissues have never been observed entirely girdling the stem; however, they frequently cause a cessation or retardation of growth and an abnormal light-green coloration of the foliage. Frequently the foliage of plants carrying stem cankers develops a reddish-bronze coloration typical of that produced by maturity in the fall. An orange-red bacterial exudate frequently occurs on these stem cankers (pl. 2, *E*).

VARIETAL SUSCEPTIBILITY

Arnaud (*1*) noticed that of two ivies under observation, one, "Lierre des Bois", was more severely attacked than the other, "Lierre d'Ecosse." The disease has been observed occurring naturally on *Hedera helix* and its varieties *baltica*, *gracilis*, *lucida*, *digitata*, and Silver Queen. Inoculations upon these varieties as well as upon the varieties *marmorata*, *alba variegata*, *dentata variegata*, *conglomerata*, *nigra*, and *coriacea* have shown all to be susceptible.

PATHOGENICITY

The pathogenicity of the organism constantly associated with these disease symptoms has been repeatedly proved by inoculations in various parts of the host under varied conditions and by various methods. Repeated reisolations and reinoculations have been successfully made. Pure cultures are easily obtained by the usual poured-plate method. Young leaf tissues (plants kept under bell jars in a constantly moist atmosphere) are readily infected by atomizing with bacterial suspensions and show positive results in as short a time as 4 days. The tiny translucent spots appeared in 5 to 6 days, and some were 7 to 8 mm in diameter in 9 days. These lesions were in all respects similar to those found on naturally infected plants. Mature leaves or woody stems show symptoms only after longer periods, varying from 2 to 3 weeks. Such tissues are rarely infected except through wounds.

THE PATHOGENE

The cultures used for the morphological, cultural, and physiological studies were isolated from characteristic leaf lesions. Some of the lesions were the result of natural infections; others were the result of artificial inoculation with the bacteria. The pathogenicity of the several cultures used in these studies was established by successful

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infections induced by inoculation of healthy, growing ivy leaves and stems.

MORPHOLOGY

Bacterium hederae is a short rod with rounded ends, rather smaller than is usual for plant pathogenes. In culture media the rods are 0.7 to 2.7μ long by 0.3 to 0.6μ wide and occur singly or in pairs or short chains. In the host tissues they are 0.7 to 2μ long by 0.2 to 0.4μ wide. They are motile by means of one polar flagellum. Capsules are present. No spores have been found.

STAINING REACTIONS

The organism is Gram-negative; it is not acid-fast. It stains readily with all the commonly used bacteriological stains. The capsules are easily demonstrated with Ribbert's dahlia capsule stain and also with Leifson's (*4*) stain, a flagella stain which stained the capsules but only rarely the flagella of the ivy bacteria which proved to be unusually difficult to stain. However, with Casares-Gil's stain it was definitely determined that there is a single polar flagellum, usually long and often quite wavy in the part nearest the rod.

Carbol-fuchsin-stained mounts from potato-dextrose agar cultures grown at 34° C. showed single rods and chains very poorly and irregularly stained, with diameters varying from 0.3 to 0.9μ. In beef-media cultures the diameter of the bacteria is slightly greater than in potato-dextrose agar cultures.

CULTURAL CHARACTERS[5]

BEEF-PEPTONE AGAR COLONIES.—On beef-peptone agar (pH 6.8 to 7.0) the colonies of *Bacterium hederae* grow slowly. In 48 hours they are usually visible as mere points of growth, and in 4 to 5 days even well-isolated colonies are only 1 to 2 mm in diameter. In 10 to 12 days a few colonies are 5 to 7 mm in diameter, but the usual size is 2 to 4 mm. White and transparent at first, they become pale yellow, Massicot yellow,[6] and translucent. They are circular, smooth, glistening, slightly elevated, with entire margins (pl. 1, *E*). The interior has definite short concentric lines or crosshatching, which disappears when the colonies are 10 to 12 days old. Beef is not a very favorable medium for this organism, and the colony characters vary with even slight differences in the media, the acidity, the moisture, the temperature, or other factors. In one set of plates the colonies had definite white halos. In another set from 3 to 20 points of secondary growth appeared within each colony. Sometimes the centers are opaque with translucent crosshatched borders, or they may be granular, mottled, or homogeneous. Buried colonies are spherical to lenticular, opaque to translucent. The growth is slightly viscid. Another fact noted is that growth in transfers from beef-media cultures is very uncertain. Unless the beef culture is young and rather heavy inoculations are made from it, more often than not no growth develops in the transfers.

BEEF-PEPTONE AGAR SLANTS.—Growth is slow and never becomes even moderately heavy. If the inoculation is from a liquid culture the resulting growth is most likely to be in the form of tiny isolated colonies, which eventually may coalesce. Inoculation from agar cultures gives a uniform, smooth, glistening streak, practically colorless except on the lower part of the slant, where the growth is somewhat thicker and pale yellow. Crosshatching or striae are present. The growth is slightly viscid or elastic; it does not draw out in a long thread but breaks at a length of 4 to 6 mm.

BEEF-EXTRACT AGAR.—Growth is very similar to that in beef-infusion agar, but the color is slightly deeper yellow and the growth is not viscid.

BEEF-PEPTONE BROTH.—The bacteria grow slowly in beef broth, even of most favorable pH value and at favorable temperatures. Thin, rolling clouds, best in

[5] Unless otherwise stated, all beef media were made with beef infusion and had a pH of 6.8 to 7.0. Cultures were grown at about 23° C.

[6] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D.C. 1912.

the upper layers of the liquid, appear in 2 days. Growth increases slowly for a number of days, but the clouding is never more than moderate. Irregular rims of pale yellow appear in 5 to 7 days and often become fairly wide and heavy. If cultures are undisturbed a thin pellicle forms. There is a moderate, translucent sediment, which rises in a spiral when shaken. Rims, pellicles, and sediment are viscid. Clouding persists for 5 to 6 months at room temperature.

POTATO-DEXTROSE BROTH.—In this medium, with a pH value of 5.6 to 5.8 the liquid clouds very slightly, but the surface growth in the form of rim and pellicle is heavy.

POTATO-DEXTROSE AGAR.—This medium with a pH value of 5.6 to 5.8 seems to be ideal for the ivy bacterium. In isolation plates the colonies reach a diameter of 10 to 12 mm. In tubes of slanted medium the surface is quickly covered with a thick, smooth, glistening layer of homogeneous to indefinitely mottled (under a × 6 lens) growth. The growth, practically colorless at first, becomes pale greenish yellow, Massicot yellow, or Chartreuse, and later chamois [7] or even darker. The texture of the growth is soft and butyrous. Old cultures sometimes show a trace of viscidity. The thick, smooth, translucent growth remains practically unchanged for weeks. The pH of the medium of cultures changes in 3 days from 5.6 to 6.6.

POTATO CYLINDERS.—On steamed potato cylinders growth at first is fairly smooth and pale yellow, but it soon becomes thin, wet, and yellowish brown with some pale yellow at the margin. The potato is moderately browned. Growth does not persist or increase. A weak diastatic action was indicated by tests with Lugol's iodine solution.

BEEF GELATIN.—In plates of beef gelatin with a pH of 7.3, the colonies were barely visible in 2 days at 20° to 23° C. In 5 days the gelatin immediately below the colonies was liquefied. In 7 days the medium of thickly sown plates was entirely liquefied and the small, spherical, compact colonies were floating in the unclouded liquid. In tube cultures slight liquefaction is evident in 2 days. In 6 to 7 days there is a 5- to 7-mm stratiform layer of liquid. Further advance is slower, 5 to 6 weeks being required for liquefaction of the entire 10 cc of medium. The liquefied gelatin is almost entirely clear. Irregular rims and thin pellicles of pale yellow form, and there is a moderate viscid sediment, deeper yellow and more opaque than in beef-broth cultures.

BLOOD SERUM.—On Loeffler's solidified blood serum, growth was doubtful for several days. Later the whole slant was covered with a smooth layer of mustard-yellow growth. No trace of liquefaction was observed until after 4 weeks, when the slanted part became translucent and yellowish. In 7 weeks some cultures were entirely liquefied, while in others only the slanted portion was liquefied. Cultures were now getting dry, and no further change occurred.

REDUCTION OF NITRATES.—Growth is fairly good in nitrate-beef bouillon, but 9-day-old cultures with the starch-iodine-sulphuric acid test showed no trace of nitrate reduction. Cultures in a synthetic nitrate medium tested when 10 days old with the α-naphthylamine sulphanilic-acid as recommended in the Manual of Methods (*7*), gave positive indications of a moderate reduction of nitrate, less than half as much as in control cultures of *Bacillus phytophthorus* and *B. aroideae*.

DIASTATIC ACTION.—Plates of beef agar plus 0.2 percent of starch were heavily inoculated with surface streaks. Growth was not vigorous. On the eighth and tenth days a partially cleared zone 15 to 20 mm wide appeared when the plates were flooded with iodine solution. Potato-cylinder cultures 5 weeks old tested with iodine also gave indication that the starch is only partially hydrolyzed.

COHN'S SOLUTION.—Repeated trials with light and with heavy inoculations show that the organism does not grow in this medium.

USCHINSKY'S SOLUTION.—When heavily inoculated a slight milky color and thin clouding appeared after 6 to 10 days. In 4 to 6 weeks the clouding was moderate and fairly heavy and pale yellow rims and pellicles formed. There was no color change in the medium.

FERMI'S SOLUTION.—Growth is slow and slight in this medium. Heavily inoculated cultures 3 weeks old are faintly clouded and have a few slender white threads suspended in the liquid or attached to the tube wall. There is no rim of pellicle and no color change in the medium.

TOLERATION OF SODIUM CHLORIDE.—In beef broth containing 1 percent of NaCl, growth is as good as in plain broth. Growth is slightly retarded by 2 percent, greatly retarded by 3 percent, and entirely lacking in 4 percent of NaCl.

INDOL PRODUCTION.—Indol is not produced. Cultures in a 2-percent peptone solution and in 1-percent tryptophane solution grow better than in beef broth.

[7] RIDGWAY, R. See footnote 6.

The tests were made with sulphuric acid and sodium nitrite. Control cultures of *Bacillus coli* produced indol.

HYDROGEN SULPHIDE PRODUCTION.—A slight amount of hydrogen sulphide is produced. Tests were made in lead acetate agar; also by strips of lead acetate paper suspended over cultures.

AMMONIA PRODUCTION.—Slight amounts of ammonia are produced in beef media and in peptone-broth cultures.

MILK.—Milk is slowly coagulated. The curd remains soft and jellylike for 2 to 3 weeks, then becomes more compact. Casein is slowly digested, about 3 months being required for complete digestion. The whey is yellowish and viscid. Numerous tyrosin crystals form in all milk cultures.

LITMUS REDUCTION.—Lavender-colored litmus-milk shows slight to no bluing. Reduction of the litmus begins in 4 to 8 days and is complete in 6 to 12 days.

METHYLENE BLUE REDUCTION.—Methylene blue in milk is considerably reduced in 2 and entirely reduced in 8 days.

FERMENTATION OF CARBOHYDRATES.—The ability of the bacteria to ferment carbohydrates was tested on peptone-free synthetic agar (*7*), with brom-cresol purple as an indicator. One percent each of dextrose, sucrose, lactose, maltose, mannite, and glycerin was used. Acid without gas was formed very promptly from dextrose and sucrose, rather slowly from lactose, and very slowly from glycerin. In maltose there was only a trace of growth and no acid reaction. There was no growth in mannite.

TEMPERATURE RELATIONS.—The optimum temperature for growth is between 20° and 26° C. Beef-bouillon cultures cloud more readily at 25° to 26° than at 20° to 22°, but after several days the cultures at the lower temperatures have the better growth. The minimum temperature for growth is 2° or lower. The maximum for beef-bouillon cultures is 32°. (Beef-bouillon cultures cloud thinly at 33° and 34° in 1 to 2 days, but this clouding disappears in 1 day or less.) Slight but persistent growth occurs on beef agar at 34° and on potato-dextrose agar at 35° and 36°. No growth occurred at 37°. The thermal death point is near 52°.

EFFECT OF FREEZING.—Potato-dextrose agar cultures held at 4° to 8° F. (−20° to −22° C.) for 4½ months, except for several short intervals of partial or complete thawing, were not killed or even noticeably reduced in vitality.

EFFECT OF SUNLIGHT.—Freshly inoculated plates of beef agar with half of each plate covered to exclude direct light were exposed for 10, 20, and 30 minutes to direct sunlight at midday, April 5, 1932. A slight haze but no clouds slightly reduced the light. In these tests the bacteria were killed in the areas subjected to the direct rays of the sun, and also to a considerable distance under the covered parts. Colony numbers were normal only in the area farthest from the light. In a repetition of the experiment at midday, April 12, 1932, when there was no haze, all bacteria were killed by exposure for 10 minutes; in 6 minutes 80 to 90 percent were killed, and in 3 minutes 40 to 60 percent were killed.

OPTIMUM REACTION AND TOLERATION LIMITS.—Because of the slow growth of the bacteria the pH values of the medium may change somewhat before the cultures cloud. Repeated tests, with the pH values of the media determined at inoculation time and again when growth became evident, indicate 7.0 as the optimum for growth, and 5.5 and 8.5 as the limits. The different strains studied varied slightly, and at different times the same strain sometimes showed slight variations in the pH requirements.

RELATION TO OXYGEN.—The organism is aerobic. There was no clouding in the closed ends of fermentation tubes containing beef bouillon and synthetic media plus carbohydrates, nor in agar tubes either with or without carbohydrates.

VITALITY IN CULTURE.—In culture media, particularly in beef media, the vitality of the ivy bacterium shows a lack of uniformity. At room temperature some beef cultures have remained alive for 7 months, but most cultures die within 2 to 8 weeks. Many transfers to beef media, especially if made from liquid culture, fail to establish any growth. On potato-dextrose agar there is no difficulty in securing vigorous growth, and no reduction in vitality has been noted in cultures of various ages up to and including some 9 months old. At temperatures below freezing, the organism remains alive at least 4 months on potato-dextrose agar. Cultures that grew slightly at 34° and 35° C. and remained at these temperatures for 20 to 30 days were to some extent reduced in vitality, as shown by growth in transfers under favorable conditions but by failure to grow under slightly adverse conditions.

EFFECT OF DESICCATION.—Tests for resistance to drying were made by placing drops from beef-bouillon cultures or growth from agar cultures diluted in water or broth on cover glasses, and after these had dried, introducing them at intervals

into culture media. As in transfers from beef cultures, there was a lack of uniformity in growth results. A large number of covers failed to give any growth after drying even a few days. Some, however, produced typical growth after drying for 43 days. If the covers were put into beef bouillon, growth very seldom developed. The best method found was to embed the cover partially in very moist potato-dextrose agar.

HISTOLOGY

Thin, stained sections of ivy leaves collected 9 days after inoculation by spraying with suspensions of *Bacterium hederae* show numerous small infected areas on the lower side of the leaf. Bacteria are abundant in these lesions, most of which have penetrated only a short distance; others extend through half the thickness of the leaf and spread laterally an equal distance. In these sections the lesions are advanced to a stage where the lower epidermal cells are broken or distorted, and cases of distinct stomatal infection have not been seen. Stomata are present on the lower surface only of the ivy, and since infection starts on the lower side of leaves inoculated by spraying, it seems more than probable that the infection is stomatal. The numerous and large intercellular spaces in these leaves afford good accommodations for masses of bacteria. First the bacteria occupy the intercellular spaces; later the cell walls break. In some cases the bacteria seem to be inside intact cell walls. In sections of older lesions there is considerable breaking down of cell walls, the bacteria occupy large pockets, and the upper as well as the lower epidermis is destroyed. Sections of leaves collected 48 hours after inoculation show no sign of infection. The bacteria in the lesions are 0.7 to 2μ by 0.2 to 0.4μ wide. Capsules were stained on rods direct from leaf lesions. Staining of flagella on leaf-lesion rods was not attempted.

TECHNICAL DESCRIPTION

Bacterium hederae Arnaud is a short, motile rod, 0.7 to 2.7μ by 0.3 to 0.6μ, with a single polar flagellum. Capsules are produced, but no spores. It is Gram-negative and is not acid-fast. It is aerobic. Gelatin and blood serum are slowly liquefied. Nitrate is slightly reduced, but no gas is produced. Diastatic action is slight. Acid without gas is formed from dextrose, sucrose, lactose, and glycerin. The organism does not form acid in milk, but reduces litmus and methylene blue in this medium. Indol is not formed. Slight amounts of hydrogen sulphide and ammonia are produced. Its optimum pH value for growth on beef media is 7.0, and the optimum temperature for growth is 20° to 26° C., maximum 36°, minimum below 2°. Its thermal death point is 52°. On beef agar growth is moderate to slight. Colonies are round, smooth, pale yellow. On potato-dextrose agar growth is abundant, pale yellow. It causes leaf spots and stem cankers on English ivy, *Hedera helix*, and its horticultural varieties.

SYNERGISM

With the appearance of Burkholder and Guterman's (*2*) report of synergism between *Bacterium hederae* and an associated organism, experiments were conducted with 10 associated bacteria which the present writers had isolated and designated as cultures *d* to *m*. Without exception these associated organisms were isolated from old spots on foliage. Isolations from young diseased areas never failed to yield pure cultures of *Bact. hederae*.

Inoculations were made on leaves injured by needle pricks with *Bacterium hederae* in pure culture and also mixed with each of the 10 associated bacteria. In one case when culture *d* was mixed with *Bact. hederae* a distinct increase in size of the infected area resulted, which was still evident after 4 weeks from the time of inoculation. In all other nine mixed inoculations (*Bact. hederae* plus cultures *e–m*), the exact reverse process took place, or a case of anergism. The spots resulting from these mixed inoculations after 1 month were approximately 2 mm in diameter and lacked the water-soaked margins characteristic of active invasion of tissue. Inoculations with *Bact. hederae* in pure culture produced spots 7 mm in diameter, and with *Bact. hederae* and culture *d* 12 mm in diameter in the same time (pl. 1, *F*).

Organisms *d* and *k* are not the associated organisms reported by Burkholder and Guterman (*2*). Their identity is unknown. The anergistic action of nine other associated organisms (*e* to *m*, inclusive) is worthy of note.

CONTROL MEASURES

As measures of control of this disease, all soil, sand, or cinders on which an infected lot of plants have stood should be removed and the beds or benches sterilized by washing or spraying with formaldehyde solution 1:50 or corrosive sublimate 1:1,000, before placing another lot of potted cuttings on them. If the infection on any lot of plants is slight, hand-picking of the infected foliage, the pruning out of all tip infections, and the discarding of plants showing stem cankers can be resorted to and will lessen the danger of increasing severity. Keeping the temperatures of the houses at 50° F. or below has also seemed to check the rapidity of spread. Excessive syringing of the plants should be avoided, as this practice tends to spread the disease, particularly from plant to plant in the same bench.

The use of protective sprays on ivy is not desirable, because of the residue deposited by them on the foliage. Potassium permanganate has been tried in concentrations as high as 1:600. At this concentration some injury took place, but injury was absent at a concentration of 1:800. The grower felt that considerable advantage from the standpoint of disease control was obtained after repeated applications, although no unsprayed plants were held as checks against these sprays. Mercuric bichloride at a concentration of 1:1,000 has also been used, and this caused slight injury to the young foliage.

Preliminary tests were made with proprietary organic-mercury sprays containing as the toxic ingredient ethyl mercury arsenate and phenyl mercury acetate in concentrations of 1:600 to 1:50. A single application of these materials at any of the concentrations used caused no perceptible injury, but with subsequent applications the foliage became yellowish and in some cases the young foliage became curled and crinkled. As a result of this injury these materials were not used in further control tests.

SUMMARY

A bacterial disease of English ivy, *Hedera helix* L., causing a leaf spot and stem canker is described. From the results of inoculations on 12 horticultural varieties, all were found susceptible. The incubation period varied from 5 to 21 days, depending on the temperature,

humidity, and age and type of tissues inoculated. Young tissues are more easily infected than old ones, and the incubation period is proportionately shorter.

The causal organism (*Bacterium hederae* Arnaud) has been isolated and its pathogenicity proved by numerous inoculation experiments. Wounds are not necessary for infection, which is evidently stomatal.

The morphological, cultural, and physiological studies of the pathogene are described in detail and a technical description is given. The index number is 5322–3115–1222.

Synergism with 1 associated bacterial organism and anergism with 9 other associated bacterial organisms were noted.

Various methods of control are suggested.

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GROWTH OF CHICKENS AS A FUNCTION OF FEED CONSUMPTION [1]

By HARRY W. TITUS, *biological chemist*, MORLEY A. JULL, *senior poultry husbandman*, and WALTER A. HENDRICKS, *junior biologist, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

The relationship between the weight of feed consumed by a growing animal and the resulting gain in live weight is one of both practical importance and theoretical interest. As a result of a large number of scattered observations, much information has been obtained regarding the factors which influence the economy of gains made by domestic animals, but the information so obtained has never been fully organized. Students of animal nutrition have realized for a long time the need of more exact knowledge of this kind; nevertheless it was not until about 10 years ago, when Spillman (*4*) [2] suggested the use of the equation of the curve of diminishing increment for expressing the relationship between feed consumption and live weight, that, from the point of view of the present writers, the first real advance was made.

In previous papers Jull and Titus (*3*) and Titus (*5*) showed that in crossbred chickens and ducks the equation of the curve of diminishing increment expresses with a high degree of accuracy the relationship between feed consumption and live weight over an appreciable interval of growth. The data presented in these earlier papers, however, did not constitute a rigorous test of the relationship because the same diet was not fed from the first feeding until the conclusion of the experiment, and because a sufficiently long interval of growth was not investigated. Furthermore, as a result of the small number of data involved and the lack of a wholly suitable method of fitting the equation, considerable doubt remained as to the true significance of some of the parameters of the equation.

The experiment described in the present paper was planned for the purpose of (1) obtaining much more extensive and critical data, (2) studying the effect of sex on the utilization of feed, and (3) determining the effect of different levels of feed intake on the relationship between feed consumption and live weight. The plan of the experiment provided for (1) feeding the same diet, but at different graduated levels of intake, to 7 pens of males, as well as to 7 pens of females, for a period of 52 weeks; (2) collecting the pertinent data on feed consumption, live weight, and mortality; and (3) making a mathematical analysis of the data.

EXPERIMENTAL MATERIAL AND METHODS

Approximately 560 chicks were hatched in electric incubators February 13, 1930, at the United States Animal Husbandry Experiment Farm, Beltsville, Md. The eggs were obtained from a flock of

[1] Received for publication Jan. 15, 1934; issued July, 1934.
[2] Reference is made by number (italic) to Literature Cited, p. 835.

Barred Plymouth Rock females mated to Rhode Island Red males. This mating was employed in order to make use of the sex-linked barring factor which enables one to separate the sexes at hatching time. After removal of the weak and otherwise unsuitable chicks, there remained 265 males and 244 females. These were distributed among 14 pens so that there were 7 pens containing 37 males each and 7 pens containing 34 females each; the remaining chicks were discarded.

The chicks were brooded under electrically heated brooders in a series of pens in a hot-water-heated brooder house. After they were a few days old they were allowed the freedom of small run yards adjoining the pens. By means of this arrangement the chicks had access to direct sunlight whenever the weather permitted. The floors of the pens and run yards were constructed of concrete.

Approximately 10 percent of the chicks developed perosis (*7*, *8*) or other abnormalities and were killed as soon as such abnormalities were observed. During the course of the experiment, as the chicks became larger, others were removed to prevent crowding in the pens.

THE DIET

The following diet, to which 1.5 percent of cod-liver oil was added, was fed in the form of a dry mash to all 14 pens of chicks from the first feeding until the end of the experiment.

	Percent
Yellow corn meal	40. 0
Ground wheat	22. 0
Corn gluten meal	10. 0
Dried buttermilk	10. 0
Meat scraps (55 percent protein)	10. 0
Special steamed bone meal	3. 0
Alfalfa-leaf meal	2. 5
Yeast preparation [3]	2. 0
Common salt	. 5
Total	100. 0

This diet, as the average of a number of analyses of different lots of it showed, contained approximately 10.4 percent of moisture, 7.1 percent of ash, 4.9 percent of ether-extractable material, 19.4 percent of crude protein, 2.6 percent of crude fiber, and 55.6 percent of nitrogen-free extract.

LEVELS OF FEED INTAKE

One pen of males and 1 pen of females were given all the feed they would eat, and the other 6 pens of each sex were fed at different lower levels of feed intake. Accurate feed-consumption data obtained over an extended period were not available for this particular cross-breed of chickens. Accordingly, in order to determine how much feed to give to each of the 12 pens of chicks that were to be kept on the lower levels of feed intake, use was made of data previously obtained by the writers with several groups of Rhode Island Red chicks in which the sexes had not been separated. From the average ad libitum feed consumption of these chicks, tables were prepared which gave the weight of feed, per chick, for each day in order that the relative levels of feed intake for the 6 pens of each sex would be

[3] A commercial yeast preparation made by drying a suspension of yeast on corn meal; it contains approximately 30 percent, by weight, of dried yeast.

87.5, 75.0, 62.5, 50.0, 37.5, and 25.0 percent, respectively, of this arbitrarily chosen standard.

The quantity of feed consumed, per chick, by the pen of females which was allowed to eat all the feed that it desired was approximately equal to the ad libitum feed consumption of the Rhode Island Red chicks mentioned previously, but the quantity consumed, per chick, by the corresponding pen of males was appreciably greater. Thus, although the relative levels of feed intake (table 1) were not the same for the corresponding pens of males and females, the absolute levels (actual quantity) of feed intake were practically the same, except for the pen of males and the pen of females which were given all they would consume.

TABLE 1.—*Levels of feed intake of the 7 pens of males and the 7 pens of females*

Males			Females		
Pen no.	Level of feed intake as percentage of the average ad libitum feed consumption of Rhode Island Reds [a]	Relative level of feed intake, as percentage [b] of the average ad libitum feed consumption of crossbred males	Pen no.	Level of feed intake as percentage of the average ad libitum feed consumption of Rhode Island Reds [a]	Relative level of feed intake, as percentage [b] of the average ad libitum feed consumption of crossbred females
	Percent	*Percent*		*Percent*	*Percent*
84	127.5	100.0	85	102.2	100.0
86	87.5	68.6	87	87.5	85.4
88	75.0	58.9	89	75.0	73.5
90	62.5	49.0	91	62.5	60.9
92	50.0	39.2	93	50.0	49.2
94	37.5	29.4	95	37.5	36.8
96	25.0	19.6	97	25.0	24.5

[a] Sexes not separated.
[b] Levels of feed intake were computed each week, and the averages for the first 36 weeks were taken.

The females in pens 85, 87, 89, and 91 began laying during the twentieth, twenty-third, twenty-fourth, and twenty-seventh weeks, respectively, of the experiment, and for a time an attempt was made to "correct" the feed consumption by subtracting 40 grams of feed for each egg laid (*6*). By the thirty-sixth week the egg production had increased to such an extent in pens 85, 87, and 89 that the writers believed it was no longer possible to "correct" the feed consumption with a sufficient degree of accuracy, and so these three pens were discontinued at the end of the thirty-sixth week.

EXPERIMENTAL DATA

Records were kept of the quantities of feed consumed per chick per week, of the live weights of the chicks at the end of each week, and of the mortality in each pen.

The experimental data on feed consumption and live weights are too voluminous for presentation here. However, the discussion centers around, and most of the conclusions depend on, a relatively small number of constants derived from the data. These constants are given in the discussion which follows.

Since mortality data are of considerable importance in interpreting the results of feeding experiments, table 2 is given. In general the

number of birds that died was very small except in the pens receiving less than 50 percent of the ad libitum level of feed.

TABLE 2.—*Number of deaths* [a] *occurring in each pen during each 4-week period*

MALES

Pen no.	Number of deaths during—													Total [b] number of deaths
	First 4 weeks	Second 4 weeks	Third 4 weeks	Fourth 4 weeks	Fifth 4 weeks	Sixth 4 weeks	Seventh 4 weeks	Eighth 4 weeks	Ninth 4 weeks	Tenth 4 weeks	Eleventh 4 weeks	Twelfth 4 weeks	Thirteenth 4 weeks	
84	0	0	0	0	1	0	0	0	0	0	0	0	0	1
86	1	0	0	0	0	0	0	1	0	0	0	0	0	2
88	1	0	0	0	0	0	0	0	0	0	0	0	1	2
90	2	0	0	0	0	0	0	0	0	2	2	0	1	7
92	0	0	0	1	0	0	0	3	3	6	2	2	0	17
94	3	1	0	0	0	1	0	1	6	6	3	0	0	21
96	17	1	0	5	2	2	3	2	2	[c] 1				35

FEMALES

85	0	0	0	2	0	0	0	0	0	[d]				2
87	0	0	0	0	0	0	0	1	0	[d]				1
89	1	0	0	0	0	0	0	1	0	[d]				2
91	1	0	0	1	0	0	0	0	0	0	0	0	0	2
93	1	0	0	0	0	2	0	0	0	0	1	0	0	4
95	0	0	1	0	0	0	0	0	3	2	1	2	1	10
97	9	0	0	2	0	2	1	1	7	[e] 7				29

[a] Exclusive of perosis, which does not directly affect growth.

[b] There were 37 chicks in each pen of males and 34 chicks in each pen of females at the beginning of the experiment.

[c] This pen was discontinued at the end of the thirty-eighth week because of excessive mortality.

[d] This pen was discontinued at the end of the thirty-sixth week because the egg production had increased to such an extent that as far as the relation between feed consumption and growth was concerned, the data were of questionable value.

[e] This pen was discontinued at the end of the thirty-seventh week because of excessive mortality.

FITTING THE EQUATION OF THE CURVE OF DIMINISHING INCREMENT TO THE EXPERIMENTAL DATA

The equation of the curve of diminishing increment was fitted to the feed-consumption and live-weight data obtained from each of the 14 pens. This equation may be written in two ways, of which one is

$$W = A - BR^F \qquad (1)$$

in which, according to Spillman's hypothesis (*4*),

W = the live weight for any corresponding feed consumption, F;
A = the maximum live weight attainable;
B = the difference between A and the initial live weight, i.e., the total gain in live weight possible;
R = the Spillman ratio, which is the inverse ratio of the gains in live weight resulting from any two successive units of feed consumed (thus, if one unit of feed produces a gain of 0.30 kilogram and the next a gain of 0.27 kilogram,

$$R = \frac{1}{\frac{0.30}{0.27}} = \frac{0.27}{0.30} = 0.9), \text{ and}$$

F = the cumulative feed consumption.

Another form in which this equation may be written is

$$W = A - B\epsilon^{-kF} \tag{2}$$

in which W, A, B, and F have the same significance as before, ϵ is the base of the natural system of logarithms, and k is a constant which is related to R by the following equation:

$$R = \epsilon^{-k} \tag{3}$$

The first step in fitting this equation to the data for each pen was to determine the approximate values of the parameters A, B, and k by the rapid method recently described by Hendricks (*1*). After having obtained the approximate values of A, B, and k, the writers employed the following adjustment equation to determine the corrections to be made to the approximate values of the parameters:

$$\frac{\partial f}{W_o \partial A_o}\alpha + \frac{\partial f}{W_o \partial B_o}\beta + \frac{\partial f}{W_o \partial k_o}\kappa = \frac{W - W_o}{W_o} \tag{4}$$

in which

$$\frac{\partial f}{\partial A_o} = 1, \quad \frac{\partial f}{\partial B_o} = -\epsilon^{-k_o F}; \text{ and } \frac{\partial f}{\partial k_o} = FB_o\epsilon^{-k_o F}$$

and α, β, and κ are corrections to be made to A_o, B_o, and k_o, respectively, which are approximations, previously obtained, of the constants A, B, and k; and W and W_o are, respectively, the corresponding observed and calculated live weights.

The corrected values of A, B, and k were readjusted until the corrections became negligible. In most cases only a single adjustment of the parameters was required because the rapid method gave values of the parameters which required only small corrections. The values of R were then calculated by means of the relationship between k and R, i.e., $R = \epsilon^{-k}$. The unit of feed weight, as well as the unit of live weight, used in this study is the kilogram. In applying the various equations, all weights should be expressed in kilograms.

The writers consider the adjustment equation used in this paper, i.e., equation 4, to be superior to the one previously used by Jull and Titus (*3*), for when equation 4 is used, the sum of the squares of the relative residuals is reduced to a minimum, whereas when the other equation is used, the sum of the squares of the absolute residuals is reduced to a minimum.

THE PARAMETERS AND DERIVED CONSTANTS

In order to summarize as briefly as possible the chief numerical results of fitting the equation of the curve of diminishing increment to the data on feed consumption and live weight, the parameters of this equation, as well as several derived constants, have been tabulated in table 3.

TABLE 3.—*Values of the constants of the live weight-feed consumption equations (curve of diminishing increment) and the coefficients of deviation of the observed from the calculated live weights for each of the 14 pens of chicks*

MALES

Pen no.	Relative level of feed intake [a]	A	Probable error of A	B	Probable error of B	k	Probable error of k	R [b] (or ϵ^{-k})	kB [c]	Probable error of kB	Coefficient of deviation [d]
	Percent	*Kilograms*	*Kilogram*	*Kilograms*	*Kilogram*						*Percent*
84	100.0	3.677	±0.031	3.642	±0.030	0.0925	±0.0015	0.9116	0.337	±0.003	4.52
86	68.6	2.956	±.010	2.923	±.010	.1203	±.0007	.8867	.351	±.001	1.56
88	58.9	2.621	±.013	2.588	±.013	.1387	±.0012	.8705	.359	±.002	2.45
90	49.0	2.235	±.010	2.202	±.010	.1627	±.0013	.8499	.358	±.002	1.99
92	39.2	1.654	±.012	1.620	±.012	.2041	±.0024	.8154	.331	±.002	3.00
94	29.4	.985	±.009	.951	±.009	.3719	±.0074	.6894	.354	±.005	5.24
96	19.6	.638	±.009	.606	±.009	.5159	±.0141	.5970	.312	±.005	4.62

FEMALES

85	100.0	2.165	±0.023	2.131	±0.022	0.1598	±0.0036	0.8523	0.341	±0.005	4.40
87	85.4	2.362	±.027	2.330	±.027	.1489	±.0031	.8617	.347	±.004	3.72
89	73.5	2.163	±.026	2.132	±.026	.1681	±.0033	.8452	.358	±.003	3.53
91	60.9	1.974	±.009	1.941	±.009	.1800	±.0015	.8353	.349	±.002	2.15
93	49.2	1.641	±.006	1.608	±.006	.2190	±.0014	.8033	.352	±.001	1.64
95	36.8	1.233	±.010	1.201	±.009	.2804	±.0033	.7555	.337	±.002	2.99
97	24.5	.645	±.008	.613	±.008	.4778	±.0095	.6201	.293	±.003	3.22
General mean [e] of kB for the 14 pens									[f].347	±.001	

[a] The level of feed intake is expressed as the percentage of the ad libitum feed consumption.

[b] The numerical value of ϵ^{-k} is equal to the inverse ratio of the gains in live weight resulting from any 2 successive units of feed consumed. This ratio is referred to as the Spillman ratio. Since $R=\epsilon^{-k}$, the probable error of k is also the probable error of $ln\ 1/R$.

[c] The numerical value of kB is equal to the maximum efficiency of the feed in producing gains in live weight. It is the value of the left-hand member of $dW/dF=kB^{-kF}$ when $F=0$.

[d] The coefficient of deviation of the observed live weights from the calculated live weights. The coefficient of deviation is analogous to the familiar coefficient of variation. In this case it was calculated by means of the formula

$$\text{C. of D.}=\sqrt{\frac{\Sigma\left[\frac{100(x-x_o)}{x_o}\right]^2}{N-3}},$$

in which x represents an observed value and x_o represents the corresponding calculated value. To show the analogy between the 2 a comparison may be made of the formula for the coefficient of deviation in the case of a 1-constant equation with that for the familiar coefficient of variation. The formula of the former is

$$\text{C. of D.} = \sqrt{\frac{\Sigma\left[\frac{100(x-x_o)}{x_o}\right]^2}{N-1}},$$

and the formula of the latter may be written

$$\text{C. of V.} = \sqrt{\frac{\Sigma\left[\frac{100(x-\bar{x})}{\bar{x}}\right]^2}{N-1}}, \text{ since C. of V.} = \frac{100\sigma}{\bar{x}} \text{ and } \sigma = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N-1}}.$$

[a] Weighted on the basis of the probable errors.
[f] If the values of the maximum efficiency of the feed in the case of pens 96 and 97 are omitted, the general mean maximum efficiency of the feed becomes 0.350±0.001.

INTERPRETATION AND DISCUSSION OF THE EXPERIMENTAL RESULTS

RELATIONSHIP BETWEEN FEED CONSUMPTION AND LIVE WEIGHT AS EXPRESSED BY EQUATION OF CURVE OF DIMINISHING INCREMENT

A comparison of the observed live weights with those calculated by means of the equation of the curve of diminishing increment showed that this equation describes the relationship between feed consumption and live weight with a high degree of accuracy.

To illustrate graphically the excellent agreement between the observed and calculated live weights, the observed average live weights

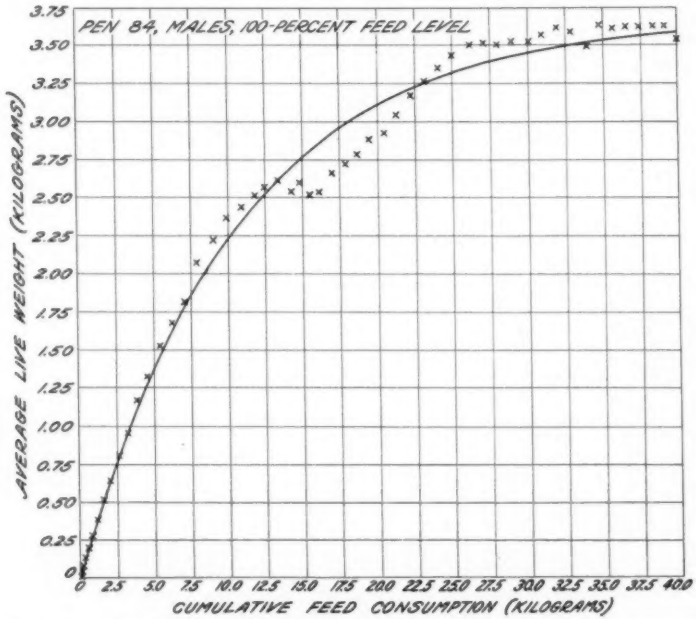

FIGURE 1.—A representative example of one of the poorer fits of the equation of the curve of diminishing increment to the experimental data. The smooth curve was plotted by means of the equation

$$W = 3.67695 - 3.64207 e^{-0.032463F} \text{ (kg)}.$$

The crosses represent observed average live weights plotted against cumulative feed consumption.

of the cockerels in pens 84 and 86 are plotted, together with the fitted curves, in figures 1 and 2, respectively. Pen 84 was selected as being representative of one of the poorer fits of the equation to the experimental data, whereas pen 86 was selected as being representative of one of the better fits. In the case of pen 84 some of the deviations of the observed live weights from the curve are rather large, but in no instance do they exceed 12 percent of the corresponding calculated values, and the coefficient of deviation is only ± 4.52 percent. In the case of pen 86 the largest deviation is less than 7 percent, and the coefficient of deviation has the very low value of ± 1.56 percent.

The coefficients of deviation of the observed from the calculated live weights for all 14 pens are given in table 3. The low values of these coefficients are further evidence of the close agreement between the observed and the calculated live weights, and hence of the ability of the equation of the curve of diminishing increment to express accurately the relationship between live weight and feed consumption.

It was of considerable interest to note that for those pens in which egg production did not complicate the picture most of the deviations, when expressed in percentage, were rather small, except in the case of the 2 pens in which the chicks were allowed to eat all they wanted and the 4 pens on the 2 lowest levels of feed intake. This at least indicates the practicability, as well as the desirability, of controlling, according to a predetermined schedule, the feed intake of animals in comparative feeding experiments, so that the feed consumed will be somewhat less than they would eat of their own free will but at least 50 percent of the quantity that they would normally be expected to eat. A level of feed intake equal to approximately 70 percent of the ad libitum level is recommended because in most cases the animals receiving feed at this level of intake may be expected to eat all that is fed them unless the feed is unpalatable, and because, as is shown later, the value of the Spillman ratio for this level is not so greatly different from its value for the ad libitum level, whereas below the 70-percent level the value of the Spillman ratio decreases with increasing rapidity as the level of feed intake is decreased.

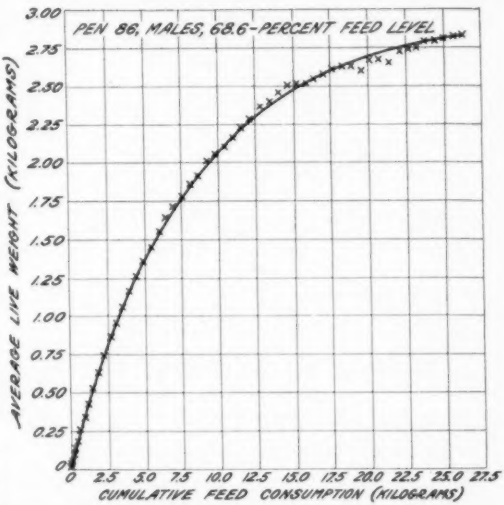

FIGURE 2.—A representative example of one of the better fits of the equation of the curve of diminishing increment to the experimental data. The smooth curve was plotted by means of the equation

$$W = 2.95629 - 2.92281e^{-0.190213F} \text{ (kg)}.$$

The crosses represent observed average live weights plotted against cumulative feed consumption.

Titus and Hendricks (*9*) have recorded the observation that when chicks are fed at different levels of intake varying from about 40 percent of ad libitum to ad libitum consumption, the live weights of less than approximately 500 grams may be expressed by a single equation relating live weight to feed consumption, regardless of the level of intake. At first the reason for this phenomenon was not clearly evident, but the present study indicates that this follows as a result of one of the properties of the equation of the curve of diminishing increment. Figures 3 and 4 illustrate this point. The curves of the fitted equations are plotted in figure 3 for males and in figure 4

for females. An examination of these curves shows that those for the levels of feed intake above 48 percent of the ad libitum feed consumption almost coincide, in the case of both the males and the females, until a live weight between 750 and 1,000 grams is reached; and for levels of feed intake above 58 percent, the curves almost coincide until a live weight of nearly 1,250 grams is reached.

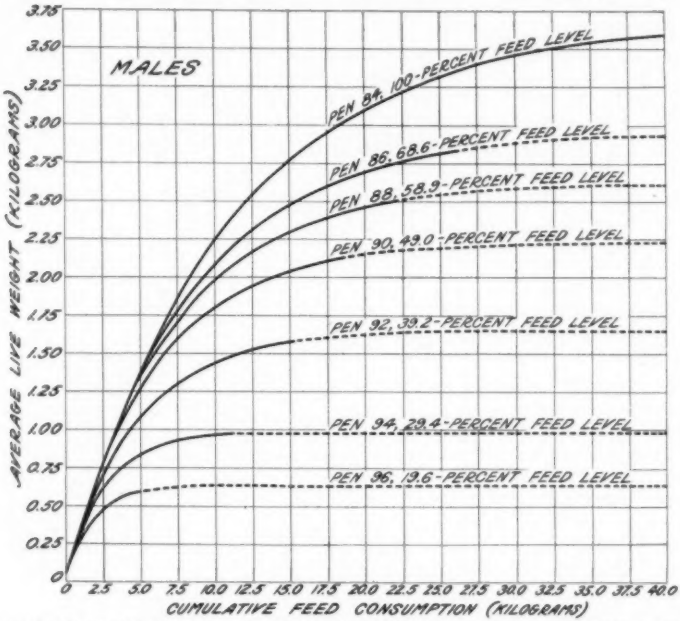
MALES
PEN 84, 100-PERCENT FEED LEVEL
PEN 86, 68.6-PERCENT FEED LEVEL
PEN 88, 58.9-PERCENT FEED LEVEL
PEN 90, 49.0-PERCENT FEED LEVEL
PEN 92, 39.2-PERCENT FEED LEVEL
PEN 94, 29.4-PERCENT FEED LEVEL
PEN 96, 19.6-PERCENT FEED LEVEL
AVERAGE LIVE WEIGHT (KILOGRAMS)
CUMULATIVE FEED CONSUMPTION (KILOGRAMS)

FIGURE 3.—Curves of the fitted equations for the males. Solid lines represent the growth interval studied; broken lines are extrapolations.

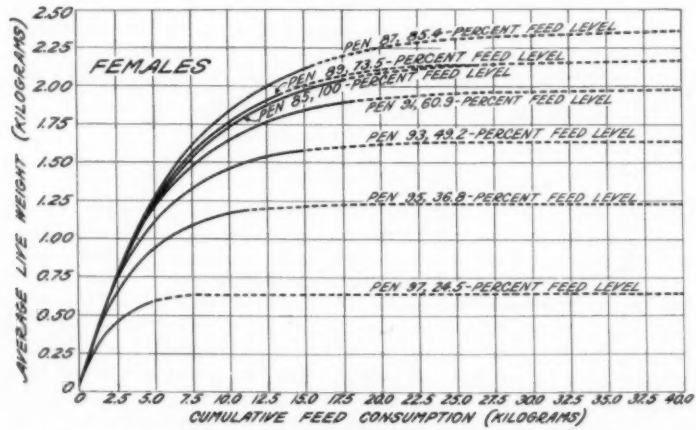
FEMALES
PEN 87, 85.4-PERCENT FEED LEVEL
PEN 89, 73.5-PERCENT FEED LEVEL
PEN 85, 100-PERCENT FEED LEVEL
PEN 91, 60.9-PERCENT FEED LEVEL
PEN 93, 49.2-PERCENT FEED LEVEL
PEN 95, 36.8-PERCENT FEED LEVEL
PEN 97, 24.5-PERCENT FEED LEVEL
AVERAGE LIVE WEIGHT (KILOGRAMS)
CUMULATIVE FEED CONSUMPTION (KILOGRAMS)

FIGURE 4.—Curves of the fitted equations for the females. Solid lines represent the growth interval studied; broken lines are extrapolations.

Having observed the close dependence of live weight on feed consumption, one may now attempt to determine the cause of the irregularities in the curves showing the relation between feed consumption and live weight (fig. 1) and between age and live weight (fig. 5) for pen 84 and between age and live weight (fig. 6) for pen 85. In figures 7 and 8 the rate of feed consumption of the males and females, respectively, is plotted against age. Inspection of these figures shows that after about the twelfth week the rate of feed consumption by the chicks in pens 84 and 85 was very irregular. And, in general, it is found that there is a direct relation between the irregularities in the live-weight curves and those in the curves depicting the rate of feed consumption. For the sake of comparison, approximations of the curves representing idealized ad libitum rate of feed consumption for males and females are shown (by broken lines) in figures 7 and 8, respectively. When the curves representing the observed rate of feed consumption are compared with the approximations of the idealized curves, the irregularities in the observed rate of feed consumption are brought out in a striking manner.

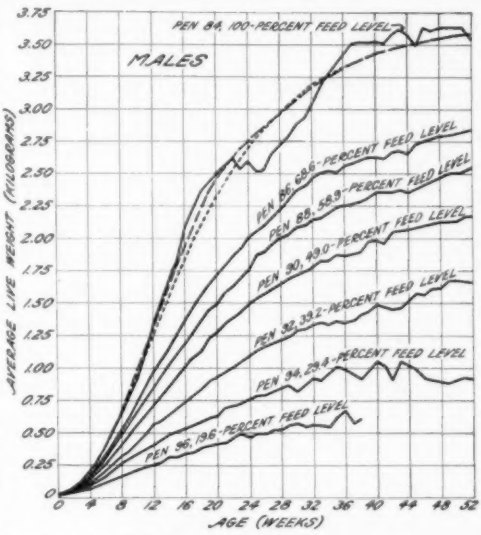
MALES
PEN 84, 100-PERCENT FEED LEVEL
PEN 86, 68.6-PERCENT FEED LEVEL
PEN 88, 58.9-PERCENT FEED LEVEL
PEN 90, 49.0-PERCENT FEED LEVEL
PEN 92, 33.2-PERCENT FEED LEVEL
PEN 94, 29.4-PERCENT FEED LEVEL
PEN 96, 19.6-PERCENT FEED LEVEL
AVERAGE LIVE WEIGHT (KILOGRAMS)
AGE (WEEKS)

FIGURE 5.—Live weights of the males plotted as a function of age. Solid lines represent observed weights; long-dash line for pen 84 was obtained by plotting, against age, the average live weights calculated by means of the equation $W=3.67695-3.64207e^{-0.0935463t}$ (kg); short-dash line for pen 84 represents the weights which would have resulted if the feed consumption had followed the curve, shown in figure 7, representing the approximation of the idealized ad libitum rate of feed consumption.

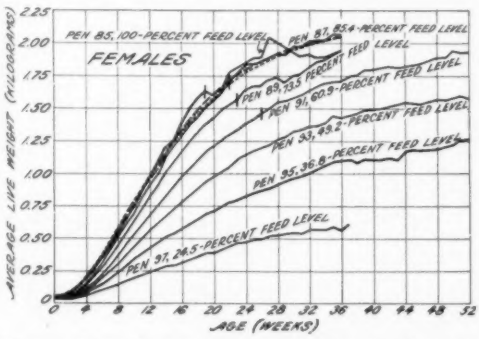
FEMALES
PEN 85, 100-PERCENT FEED LEVEL
PEN 87, 85.4-PERCENT FEED LEVEL
PEN 89, 73.5-PERCENT FEED LEVEL
PEN 91, 60.9-PERCENT FEED LEVEL
PEN 93, 49.2-PERCENT FEED LEVEL
PEN 95, 36.8-PERCENT FEED LEVEL
PEN 97, 24.5-PERCENT FEED LEVEL
AVERAGE LIVE WEIGHT (KILOGRAMS)
AGE (WEEKS)

FIGURE 6.—Live weights of the females plotted as a function of age. Solid lines represent observed weights; long-dash line for pen 85 was obtained by plotting, against age, the average live weights of the chicks calculated by means of the equation $W=2.16495-2.13054e^{-0.1396435t}$ (kg); short-dash line represents the weights which would have resulted if the feed consumption of the chicks in pen 85 had followed the approximation of the idealized ad libitum rate of feed consumption curve shown in figure 8. The short lines transecting the curves indicate the approximate age of the females, at beginning of laying, in those pens the feed-consumption data of which were corrected for egg production.

From the explanation just given of the irregularities observed in the curves showing the relation between feed

consumption and live weight, and age and live weight, it follows that in conducting a comparative feeding experiment better results are obtained if, in addition to giving all the groups of chickens the same quantity of feed per head, it is arranged that the rate of feed consumption be a fixed percentage of a carefully made approximation of the idealized rate. Obviously, uniformly regular growth can take place only when the rate of feed consumption closely follows a uniformly regular course.

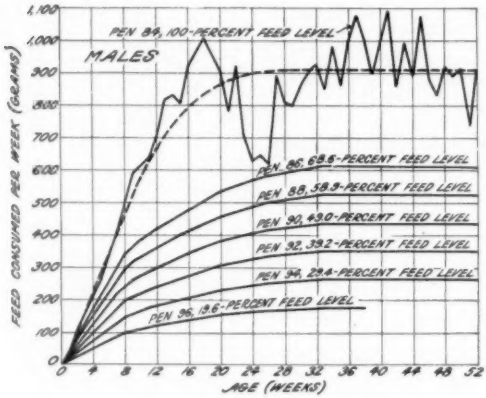
PEN 84, 100-PERCENT FEED LEVEL
MALES
PEN 86, 68.6-PERCENT FEED LEVEL
PEN 88, 58.9-PERCENT FEED LEVEL
PEN 90, 49.0-PERCENT FEED LEVEL
PEN 92, 39.2-PERCENT FEED LEVEL
PEN 94, 29.4-PERCENT FEED LEVEL
PEN 96, 19.6-PERCENT FEED LEVEL
FEED CONSUMED PER WEEK (GRAMS)
AGE (WEEKS)

FIGURE 7.—Grams of feed consumed per chick per week in the seven pens of males plotted against age. The solid lines represent the observed feed consumption per chick per week; the broken line is an approximation of the curve showing the idealized ad libitum rate of feed consumption of the males in pen 84.

EFFECT OF THE LEVEL OF FEED INTAKE ON THE NUMERICAL VALUE OF THE SPILLMAN RATIO

According to the data presented in table 3, the Spillman ratio (R in equations 1 and 3) decreases in magnitude as the relative level of feed intake is decreased. It now remains to determine the nature of the relationship between the relative level of feed intake and the numerical value of R. If these two variables are plotted against each other, as in figure 9, it at first appears that the relationship is expressible by the equation of the curve of diminishing increment. However, if a calculation is made of the values of R when the unit of feed consumption is 10 kilograms instead of 1 kilogram, and these new values are plotted against the relative levels of feed intake, the sigmoid shape of the curve at once becomes apparent.

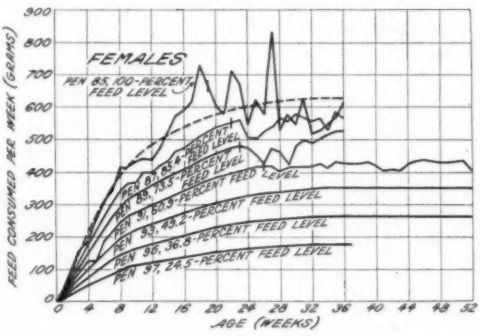
FEMALES
PEN 85, 100-PERCENT FEED LEVEL
PEN 87, 68.6-PERCENT FEED LEVEL
PEN 89, 58.9-PERCENT FEED LEVEL
PEN 91, 49.0-PERCENT FEED LEVEL
PEN 93, 39.2-PERCENT FEED LEVEL
PEN 95, 29.4-PERCENT FEED LEVEL
PEN 97, 19.6-PERCENT FEED LEVEL
FEED CONSUMED PER WEEK (GRAMS)
AGE (WEEKS)

FIGURE 8.—Grams of feed consumed per chick per week in the seven pens of females plotted against age. The solid lines represent the observed feed consumption per chick per week; the broken line is an approximation of the curve showing the idealized ad libitum rate of feed consumption of the females in pen 85. The short lines transecting the curves indicate the approximate age of the females, at beginning of laying, in those pens the feed-consumption data of which were corrected for egg production.

Because of the sigmoid shape of the curve (under the conditions just stated) it was decided to fit the equation

$$ln\frac{y}{a-y}=k(x-b) \qquad (5)$$

to the data. When this was done it was found that an excellent fit was obtained, since in no case did the observed values differ from the calculated values by more than 3 percent and the coefficient of deviation was only ±2.13 percent in the case of the males and ±0.95 percent in the case of the females. The results obtained by fitting equation 5 to the two sets of data are given in table 4.

TABLE 4.—*Effect of the level of feed intake on the numerical value of the Spillman ratio, R*

Males					Females				
Relative level of feed intake [a] (percent)	Spillman ratio, R (or ϵ^{-k})		Difference between observed and calculated		Relative level of feed intake [a] (percent)	Spillman ratio, R (or ϵ^{-k})		Difference between observed and calculated	
	Observed	Calculated [b]	Absolute	Relative		Observed	Calculated [c]	Absolute	Relative
				Percent					*Percent*
100.0	0.9116	0.9096	+0.0020	+0.22	100.0	0.8523	0.8563	−0.0040	−0.47
68.6	.8867	.8928	−.0061	−.68	85.4	.8617	.8543	+.0074	+.87
58.9	.8705	.8763	−.0058	−.66	73.5	.8452	.8499	−.0047	−.55
49.0	.8499	.8458	+.0041	+.49	60.9	.8353	.8380	−.0027	−.32
39.2	.8154	.7937	+.0217	+2.74	49.2	.8033	.8114	−.0081	−1.00
29.4	.6894	.7103	−.0209	−2.94	36.3	.7555	.7476	+.0079	+1.06
19.6	.5970	.5919	+.0051	+.86	24.5	.6201	.6222	−.0021	−.34
Coefficient of deviation.				±2.13	Coefficient of deviation.				±.95

[a] Expressed as percentage of the ad libitum feed consumption.

[b] Calculated by means of the equation, $ln\frac{y}{0.91212-y}=0.06568(x-10.2403)$, in which y=the Spillman ratio, R, and x=the level of feed intake.

[c] Calculated by means of the equation, $ln\frac{y}{0.85728-y}=0.07689(x-11.8420)$, in which y and x have the same significance as in the preceding footnote.

Figure 9 clearly shows the relationship between the Spillman ratio and the relative level of feed intake. Although, as previously stated, this ratio decreases as the level of feed intake is decreased, the rate of decrease is relatively slow until a level of about 50 percent is reached, after which it becomes very rapid. This was one of the reasons that earlier in this paper a level of feed intake equal to 70 percent of the ad libitum level was recommended for use in comparative feeding experiments.

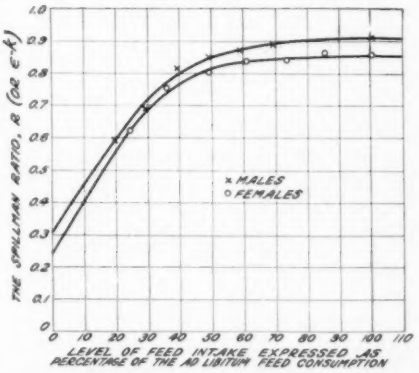

FIGURE 9.—Effect of level of feed intake on the Spillman ratio, R (or ϵ^{-k}). The solid-line curves were plotted by means of the equations given in footnotes *b* and *c* of table 4.

By means of the equations given in the footnotes to table 4 one may calculate the value of R with a high degree of accuracy for any relative level of feed intake, at least over the interval studied, i.e., between approximately 20 and 100 percent of the ad libitum feed consumption. In the case of the males it

is found that R is equal to 0.9096 for the ad libitum level and 0.8945 for the 70-percent level; and in the case of the females the corresponding values of R are 0.8563 and 0.8476, respectively.

With the exception of swine, the growing animal makes larger gains in live weight when full-fed than when underfed, and makes them more economically. Hence, two very practical questions arise: (1) What constitutes underfeeding? and (2) how serious is a given degree of underfeeding? The answer to the first question is that anything materially less than the ad libitum level of feed intake is underfeeding in the case of the growing animal. The second question can be answered satisfactorily only when the case is accurately specified. Nevertheless it may be shown, by means of the figures given in the preceding paragraph, that the first 4 kilograms of feed consumed by a growing cockerel, if fed at the 70-percent level, are utilized nearly 97 percent[4] as efficiently as they would be if fed at the 100-percent, or ad libitum, level. In the case of the growing pullet the feed is utilized slightly more than 98 percent as well at the 70-percent level as it is at the 100-percent level.

When an animal is grossly underfed, the situation is quite different. For example, a pullet utilizes her first 4 kilograms of feed only about 83 percent as well at the 40-percent level of intake as at the 100-percent level, and the cockerel utilizes his feed only about 79 percent as well.

EFFECT OF THE LEVEL OF FEED INTAKE ON THE TOTAL GAIN POSSIBLE

The data presented in table 5 show that B, the total gain possible on any given level of feed intake, decreases as the level of feed intake is decreased. This is shown graphically in figure 10, which indicates the mathematical form of the relationship between the two. Too few data are available to enable one to determine the precise form of the equation most suitable for expressing B as a function of level of feed intake; however, to enable one to estimate the numerical value of B for any relative level of feed intake within the range studied, the data were graduated by means of the equation

$$y = a - b\epsilon^{-kx} \tag{6}$$

In table 5 the numerical values of B, calculated by means of this equation, are compared with the observed values. Although the

[4] For computing the relative efficiency of utilization of the feed when fed at two different levels, a function involving only R is to be preferred to one involving both B and R, since R (table 4) was graduated with much greater precision than B (table 5). A suitable function involving only R may be obtained as follows:

Since $W = A - BR^x$, the initial weight, W_1, is given by the equation $W_1 = A - B$. It follows, then, that the gain, G, may be expressed as follows: $G = W - W_1 = (A - BR^x) - (A - B) = B(1 - R^x)$. Hence, the ratio of the gain resulting from the first 4 kilograms of feed at the 70-percent level of intake to the gain resulting from the same quantity of feed at the ad libitum level is

$$\frac{G_{70}}{G_{100}} = \frac{B_{70}\,(1 - R^4{}_{70})}{B_{100}(1 - R^4{}_{100})}.$$

Since the product of k and B is a constant, $k_{70}B_{70} =$ a constant $= k_{100}B_{100}$; hence

$$\frac{B_{70}}{B_{100}} = \frac{k_{100}}{k_{70}}.$$

Also, since $\epsilon^{-k} = R$, $k = -2.3026 \log R$. Hence, one may write

$$\frac{G_{70}}{G_{100}} = \frac{B_{70}(1 - R^4{}_{70})}{B_{100}(1 - R^4{}_{100})} = \frac{k_{100}(1 - R^4{}_{70})}{k_{70}(1 - R^4{}_{100})} = \frac{(-2.3026 \log R_{100})\,(1 - R^4{}_{70})}{(-2.3026 \log R_{70})\,(1 - R^4{}_{100})} = \frac{(-\log R_{100})\,(1 - R^4{}_{70})}{(-\log R_{70})\,(1 - R^4{}_{100})} =$$

$$\frac{(-\log 0.9096)\,(1 - \overline{0.8945}^4)}{(-\log 0.8945)\,(1 - \overline{0.9094}^4)} = \frac{(0.0411495)\,(0.3598)}{(0.0484197)\,(0.3155)} = \frac{0.01480559010}{0.01527641535} = 0.9692, \text{ or approximately 97 percent.}$$

agreement between the two is not so good as is to be desired, especially in the case of the males, one can interpolate by means of equation 6 the expected total gain for any level of feed intake with a fair degree of accuracy.

Table 5 shows that the calculated value of B for the pen of males and the pen of females which ate all the feed they wanted is appreciably greater than the observed value in both cases. This, no doubt, is due in part to the fact that the chicks in these two pens wasted some of the feed given them. The value of B for the pen of males on the 29.4-percent level of feed intake is out of line with the values of this parameter for the other pens of males; likewise the value of A, the maximum average live weight attainable, is out of line. The reason for these apparent discrepancies is not known.

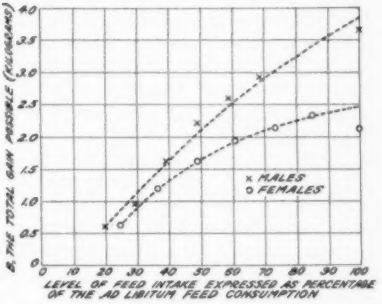

FIGURE 10.—Effect of level of feed intake on B, the total gain possible. The dash-line curves were plotted by means of the equations given in footnotes *b* and *c* of table 5.

TABLE 5.—*Effect of level of feed intake on numerical value of B, total gain possible*

Males					Females				
Relative level of feed intake [a] (percent)	B (total gain possible)		Difference between observed and calculated		Relative level of feed intake [a] (percent)	B (total gain possible)		Difference between observed and calculated	
	Observed	Calculated [b]	Absolute	Relative		Observed	Calculated [c]	Absolute	Relative
	Kilograms	*Kilograms*	*Kilogram*	*Percent*		*Kilograms*	*Kilograms*	*Kilogram*	*Percent*
100.0	3.642	3.845	−0.203	−5.28	100.0	[d] 2.131	2.459	−0.328	[e] −13.34
68.6	2.923	2.837	+.086	+3.02	85.4	2.330	2.317	+.013	+.58
58.9	2.588	2.465	+.123	+5.00	73.5	2.132	2.154	−.022	−1.04
49.0	2.202	2.050	+.152	+7.42	60.9	1.941	1.919	+.022	+1.12
39.2	1.620	1.602	+.018	+1.14	49.2	1.608	1.623	−.015	−.89
29.4	.951	1.113	−.162	−14.54	36.8	1.201	1.195	+.006	+.54
19.6	.606	.579	+.027	+4.64	24.5	.613	.613		
Coefficient of deviation				±9.37	Coefficient of deviation				[f] ±6.74

[a] Expressed as percentage of ad libitum feed consumption.
[b] Calculated by means of the equation, $y=6.97497-7.61302e^{-0.00888843x}$, in which $y=B$, the total gain possible, and x=the level of feed intake.
[c] Calculated by means of the equation, $y=2.77507-4.03429e^{-0.0154642x}$, in which y and x have the same significance as in the preceding footnote.
[d] This value of B is obviously out of line with the other values of B and, hence, was not used in fitting the equation, $y=a-be^{-kx}$, to the data for the females.
[e] When this value is omitted (for the reason stated in footnote [d]) the coefficient of deviation becomes ±1.12 percent.
[f] As stated in footnote [e], when the first value in this column is omitted, the coefficient of deviation is reduced from 6.74 to 1.12 percent.

SIGNIFICANCE OF PARAMETERS OF EQUATION OF CURVE OF DIMINISHING INCREMENT WHEN APPLIED TO DATA ON FEED CONSUMPTION AND LIVE WEIGHT

Jull and Titus (*3*), in their earlier study of the growth of chickens in relation to feed consumption, were led to the conclusion that the

parameters A and B (M and A, respectively, in their paper) could be considered only as empirical constants. In the present study a much more extensive set of data was obtained, and in the interval between the two investigations a very satisfactory and suitable method of fitting the equation was developed.

A comparison of the observed and calculated live weights showed that the average initial live weights, i.e., the average live weights before any feed was consumed, were reproduced with a high degree of precision by the equation of the curve of diminishing increment. Furthermore, this comparison showed that, on the whole, the observed and calculated average live weights agreed extremely well throughout the growth intervals studied, especially in those pens in which the feed consumption was controlled and in which the level of feed intake was more than 50 percent of the ad libitum level. These considerations lead the writers to the following conclusions regarding the significance of the three parameters of the equation of the curve of diminishing increment.

(1) A represents the maximum average live weight attainable on a given level of feed intake, provided that that level is maintained and that no appreciable fattening occurs. In the case of chickens allowed to eat all they want of an adequate diet, A represents the maximum average live weight attainable, provided no appreciable fattening occurs.

(2) B represents the difference between the maximum average live weight, A, and the average initial live weight; that is, it is numerically equivalent to the average total gain which can be made, if no appreciable fattening occurs.

(3) R, the Spillman ratio, is the inverse ratio of the gains in live weight resulting from any two successive units of feed consumed.

Since the first derivative of the equation of the curve of diminishing increment may be written

$$dW/dF = kB\epsilon^{-kF} \tag{6}$$

or

$$dW/dF = kBR^{F} \tag{6'}$$

it follows that kB is equal to the maximum efficiency of the feed in producing gains in live weight, if this efficiency is defined as the ratio of the gain in live weight to the quantity of feed required to produce the gain. The efficiency of successive units of feed decreases in geometrical progression and the magnitude of the decrease is determined by the Spillman ratio, R (or ϵ^{-k}).

In regard to its significance, kB should not be confused with kA, which the writers proposed in an earlier paper (*2*) as a measure of feed efficiency. The former represents the maximum efficiency which a feed may actually have and the latter represents the maximum efficiency which it would have if the animal to which it is fed had an inital live weight of zero. The value of kA is dependent on the maximum live weight attainable by the animal, whereas the value of kB is dependent on both the maximum and initial live weights, since

$$B = A - w_1 \text{ (w_1 being the initial live weight).}$$

The values of the maximum efficiency of the feed (kB) are given in table 3 for each of the 14 pens. Although there appears to be a slight tendency for the maximum efficiency to increase at first and then to decrease as the level of feed intake is decreased, there is a marked agreement among the values. If the values for the two pens on the lowest absolute level of feed intake are eliminated, the weighted mean value of kB is 0.350 ± 0.001 and the range is from 0.331 to 0.359.

The various numerical values of A, B, and R in the equation of the curve of diminishing increment and the parameters of the equations which are given for expressing the relationship between (1) R (the Spillman ratio) and the level of feed intake and (2) B (the total gain possible) and the level of feed intake hold only for the particular diet fed and for the particular crossbreed of chicken used. However, it is reasonably certain that, with suitable values for the parameters, the several equations used in this study will hold for any adequate diet and for any breed of chickens.

EFFECT OF SEX ON UTILIZATION OF FEED

If, for corresponding absolute levels of feed intake, one compares the numerical values of the Spillman ratio for the two sexes, he finds that for the first three of the levels less than the ad libitum level the values of R are greater for the males than they are for the females, but on the two lowest absolute levels the opposite is true. If it is assumed that the product kB is constant regardless of sex or level of feed intake (and the values of this product given in table 3 seem to indicate that this may be the case), one is led to the conclusion that on the higher absolute levels of feed intake the males of this crossbreed are more efficient in the utilization of feed than the females, but on the lower levels the latter are the more efficient.

Even if one does not make this assumption regarding the constancy of the product kB, the conclusion still appears to hold for the following reasons:

(1) The efficiency of the feed is given by the equation

$$\text{Efficiency} = dW/dF = kBR^r;$$

(2) The Spillman ratio, R, decreases at a greater rate than does the product kB after the latter's apparent maximum is reached;

(3) The values of the Spillman ratio on the higher levels of feed intake are greater for the males than for the females; and

(4) On the two lowest levels the opposite of the preceding statement is true.

If, instead of comparing the efficiency of the males and females on the basis of the absolute levels of feed intake, one makes the comparison on the basis of the relative levels of feed intake, it is found that the males are the more efficient for all the relative levels studied.

SUMMARY AND CONCLUSIONS

Extensive and critical data were obtained on the relationship between feed consumption and live weight in the case of crossbred chickens (Rhode Island Red males mated to Barred Plymouth Rock females). Seven pens of males and seven pens of females were fed

at different levels of feed intake, including the ad libitum and six lower levels, for a period of 52 weeks. The weight of feed consumed per chicken per week and the average live weight of the chickens at the end of each week were obtained.

By means of a suitable method, the equation of the curve of diminishing increment was fitted to the data recorded for each of the 14 pens of chickens. It was found that the average live weights computed by means of this equation agreed very closely with the observed average live weights. It was also found that a rational meaning could be assigned to the three parameters of this equation.

It was possible to describe accurately, by means of a simple equation, the relationship between the level of feed intake and the Spillman ratio. It is recommended that in comparative feeding experiments all the groups be fed at a level of feed intake equal to 70 percent of an approximation of the idealized ad libitum feed consumption. At this level the value of the Spillman ratio is nearly as large as it is at the ad libitum level. Under these conditions the several groups would eat the same quantity of feed, unless it were unpalatable, and the feed consumption would follow a definite course. The latter point is of importance since uniformly regular growth can take place only when the feed consumption follows a uniformly regular course.

The following conclusions are drawn:

The equation of the curve of diminishing increment, $W = A - BR^x$, describes with a high degree of accuracy the relationship between feed consumption and live weight over a wide range of levels of feed intake.

A in the above equation represents the maximum live weight attainable on a given level of feed intake, provided this level is maintained and no appreciable fattening occurs.

B in the above equation represents the maximum gain possible under the conditions just stated.

R, the Spillman ratio, is the inverse ratio of the gains in live weight resulting from any two successive units of feed consumed.

The relationship between level of feed intake and the numerical value of R is expressible, over the range of levels studied, by the equation,

$$ln \frac{y}{a-y} = k(x-b), \text{ in which}$$

y = the Spillman ratio, x = the relative level of feed intake, and a, k, and b are constants.

The relationship between level of feed intake and the numerical value of B is expressible, for the range of levels studied, with a fair degree of accuracy by the equation

$$y = a - b\epsilon^{-kx}, \text{ in which}$$

$y = B$, the total gain possible; x = the relative level of feed intake, and a, b, and k are constants.

On the higher absolute levels of feed intake the males of the cross-breed studied are more efficient than the females in their utilization of feed for growth, whereas on the very low absolute levels the opposite is true.

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COMPARISON OF THE PULLORIN AND THE RAPID WHOLE-BLOOD AGGLUTINATION TESTS FOR PULLORUM DISEASE [1]

By HUBERT BUNYEA [2]

Veterinarian, Pathological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

The control of pullorum disease is based on the diagnosis of the carrier fowl and its elimination from breeding. Various tests are at present in use in different parts of the country for diagnosing the pullorum-disease carrier. The "long" or "tube" agglutination test has been in use since 1913 and has been developed to a considerable degree of refinement. The principal objections to it are its slowness and the comparatively great expense and labor involved in its application. To meet these objections more simple methods have since been devised. Some of these retain the principle of serum-antigen agglutination, but one is based upon the allergic reaction of living tissue. This last is known as "the pullorin test" or "the intradermic test" for pullorum disease. The name "pullorin" refers to the reagent employed.

However, in the 17 years in which it has been known, the pullorin test has not been widely accepted by poultry-disease diagnosticians, many of whom have been skeptical as to its accuracy. The experiments recorded in this paper were conducted with a view to ascertaining more fully the relative diagnostic value of the pullorin test as compared with the rapid whole-blood agglutination test [3] for pullorum disease. The latter test involves the use of a stained antigen. The rapid whole-blood agglutination test was selected for the comparisons because it had the advantages claimed for the pullorin test with regard to simplicity and economy. The experiments also included some corroborative tests by the tube agglutination method.

HISTORICAL REVIEW

Ward and Gallagher [4] in 1917 first reported the development of an intradermic test for pullorum disease. These workers prepared an allergic reagent by inoculating broth with cultures of *Salmonella pullorum*, incubating at 37° C. for 1 month, holding the broth in an ice box for about 6 months, then passing it through a Berkefeld filter, and finally preserving it with 0.5-percent phenol. This product was

[1] Received for publication Mar. 30, 1934; issued July 1934.

[2] The writer expresses his indebtedness to J. M. Rosell, professeur de bacteriologie a l'Ecole de Medicine Veterinaire de la Province de Quebec et a l'Institut Agricole d'Oka, for preparing pullorin R and administering and interpreting this test in the flock in which it was used; to George W. Stiles, in charge of the U.S. Department of Agriculture branch pathological laboratory at Denver, Colo., for preparing pullorin S; and to W. J. Hall, assistant veterinarian in charge of the Department's branch pathological laboratory, Beltsville, Md., for his valuable assistance in conducting the agglutination test on the flock in which pullorins R and S were used.

[3] SCHAFFER, J. M., MACDONALD, A. D., HALL, W. J., and BUNYEA, H. A STAINED ANTIGEN FOR THE RAPID WHOLE BLOOD TEST FOR PULLORUM DISEASE. Jour. Amer. Vet. Med. Assoc. (n.s. 32) 79: 236-240. 1931.

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injected intradermically into one of the wattles of the fowl to be tested. After 24 hours the injected wattles of infected fowls were swollen, and those of uninfected fowls were not swollen. The swelling of the wattles of reactors increased noticeably in the next 24 hours.

These authors later modified their product by growing the organism in broth for 1 month at 37.5° C., and killing it by heating at 60° for 1 hour in a water bath. They then carbolized it to 0.5 percent. This product was employed without filtration.

The formula thus developed has been largely the basis of most of the commercial and experimental pullorins since used. Pullorins of the type of that first described are usually referred to as noncellular, or cell-free pullorins, whereas those of the second type are known as cellular pullorins.

A number of contemporary workers have investigated the possibilities of the diagnosis of pullorum disease by means of an allergic test. Rettger and Plastridge [5] state: "The beliefs expressed at the 1930 Poultry Congress in London were, with one exception, distinctly unfavorable to the pullorin test." A translation of Rosell's [6] comments on the comparative results between the pullorin test and the stained-antigen, rapid whole-blood test is as follows:

> In a comparative test of the efficiency of this method with those employing different kinds of pullorin, one of which was of the soluble type which I prepared by a new method, the results achieved in collaboration with Drs. Hall and Bunyea were favorable to the whole-blood method which possessed the advantage of requiring only one visit to the poultry plant. This method assuredly provides the more practical means of combating pullorum disease.

EXPERIMENTAL PROCEDURE

The experiments comprised field trials of the pullorin test in comparison with the stained-antigen, rapid whole-blood agglutination test [7] applied to previously untested poultry flocks in the vicinity of Washington, D.C. The plan of this investigation included the use of several commercial brands of pullorin and one or more pullorins prepared by research workers. Arrangements were accordingly made with four nearby flock owners for the application of the comparative tests, which were to be conducted simultaneously. However, neither test was to be set up as the standard by which to judge the merits or defects of the other. The value of a diagnostic test consists in its ability to detect the presence of the elements or processes of disease. Therefore, the criterion proposed in these experiments was that of the laboratory demonstration of *Salmonella pullorum* in the carcasses of reacting fowls selected from the four flocks tested.

In the case of fowls slaughtered from the last three flocks, a tube test was included at autopsy, as having some corroborative value with reference to the rapid whole-blood agglutination test findings.

Five different pullorins were used, two of which were noncellular and three cellular. The two experimental pullorins, R and S, were prepared, respectively, by J. M. Rosell [8] and George W. Stiles. Rosell gives the following information [9] concerning his method of preparation of the cell-free pullorin:

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[5] RETTGER, L. F., and PLASTRIDGE, W. H. PULLORUM DISEASE OF DOMESTIC FOWL. A MONOGRAPH. Conn. (Storrs) Agr. Expt. Sta. Bull. 178, pp. [109]–192 illus. 1932.

[6] ROSELL, J. M. PROGRÈS ET NORMES SUR QUELQUES POINTS D'ACTUALITÉ EN PATHOLOGIE ANIMALE. Rev. Inst. Agr. Oka 7 (3): 100–104. 1933.

[7] SCHAFFER, J. M., MACDONALD, A. D., HALL, W. J., and BUNYEA, H. See footnote 3.

[8] ROSELL, J. M. See footnote 6.

[9] ROSELL, J. M. Informal communication.

Five strains of *Salmonella pullorum* (B.A.I. strains 10, 11, 14, 17, and 20) were used in preparing the pullorin. Each strain was grown separately in flasks containing 20 cc of glucose-peptone broth adjusted to a reaction of pH 7.2. The flasks were incubated for 10 days at 37° C. At the end of this period sufficient sterile sodium hydroxide was added so that the broth gave a clear alkaline reaction, by using phenolphthalein as an indicator. It was again incubated for 3 days at 37° or 40° C. in order to obtain a better autolysis of the cells.

After the 3-day incubation period, the culture were frozen for many hours by the use of dry ice, and then melted at 45° C. This procedure of freezing and melting was repeated twice in order that a more complete autolysis be obtained. Microscopically, smears revealed that most of the cells were autolysed.

After purity tests were made the different culture autolysates were mixed and centrifuged in large centrifugal tubes. The supernatant fluid was removed by decantation. To the reunited sediments 20 cc of 0.75-percent sterile potassium hydroxide was added, and this was then shaken and heated at 50° C. until a gelatinous fluid was obtained. This fluid was then centrifuged and the supernatant fluid was added to the supernatant fluid obtained from the first centrifugation. This liquid was adjusted to a pH of 7.2 by adding N/10 hydrochloric acid.

To obtain a more concentrated pullorin the liquid was submitted to a process of evaporation without heating it over 48° C. The liquid was placed in a shallow porcelain pan which was maintained in a water bath of 48° C. for 5 hours. During this time air was allowed to pass through the liquid by means of small glass pipes, and the air currents from an electric fan passed over the surface of the liquid. By this means 1,000 cc of the fluid was evaporated to 666 cc.

Sufficient carbolic acid was then added to the product so that it would contain 0.3 percent of this preservative. It was then filtered through a Mandler candle and transferred aseptically into 20-cc sterile rubber-stoppered vials.

The pullorin supplied by Dr. Stiles was prepared by him essentially after the modified Ward and Gallagher formula, as follows: Five or six flasks of broth were inoculated with separate strains of *Salmonella pullorum*, incubated for at least 1 month, heated at 60° C. for 2 hours, carbolized, and tested for sterility. They were mixed before use.

No information is available as to the methods used in the manufacture of the several commercial pullorins.

The method employed in determining the presence of *Salmonella pullorum* infection in the slaughtered birds was the same as that used by Bunyea and Hall,[10] namely, the aseptic removal and crushing of the entire ovary or testicle, which was then placed in a culture flask of beef infusion broth containing brilliant-green dye in the proportion of 1 to 50,000. Individual colonies of *S. pullorum* were recovered from subcultures made from the brilliant-green broth cultures onto plain agar. The organisms were identified in each instance in the manner described by Bunyea and Hall.

RESULTS OBTAINED

Table 1 shows the percentage of agreements and disagreements between the reactions (1) to the various pullorins used intradermically in the pullorin test and (2) to results obtained with the rapid whole-blood agglutination test.

[10] BUNYEA, H., and HALL, W. J. THE RELATION OF AGGLUTINATION REACTION TO SALMONELLA PULLORUM INFECTION IN HENS, AND OBSERVATIONS ON THE DIAGNOSTIC EFFICIENCY OF TEST METHODS. Jour. Amer. Vet. Med. Assoc. (n.s. 33) 80: 491-496, illus. 1932.

TABLE 1.—*Percentages of agreements and disagreements between results of the pullorin test and the rapid whole-blood agglutination test*

Pullorin used		Birds tested	Agreements [1]		Disagreements [1]	
Source and designation	Type		Pullorin +, agglutination +	Pullorin −, agglutination −	Pullorin +, agglutination −	Pullorin −, agglutination +
Experimental:		*Number*	*Percent*	*Percent*	*Percent*	*Percent*
R	Noncellular	218	18.4	49.5	5.5	26.6
S	Cellular	107	23.4	44.9	12.0	19.7
Commercial:						
1	do	224	1.3	66.5	3.6	28.6
2	Noncellular	182	17.0	52.2	14.8	16.0
3	Cellular	129	2.3	71.3	6.2	20.2
Total or average		860	11.8	57.2	7.9	23.0

[1] + indicates a positive reaction; −, a negative reaction.

From table 1 it may be observed that the total average agreement in both positive and negative cases is 69 percent, whereas the total average disagreements aggregate 31 percent. Marked fluctuations for the various pullorins are plainly evident.

Tables 2 to 4 show comparisons of the results obtained from the pullorin tests, and the rapid whole-blood agglutination tests, based on their agreement or disagreement with bacteriological findings. Tables 3 and 4 include results obtained from the tube agglutination test, used post mortem.

TABLE 2.—*Comparison of results obtained from pullorins R and S and the rapid whole-blood agglutination test, based on their agreement or disagreement with bacteriological findings*

Fowl no.	Reaction of bird to—			Post-mortem findings		Agreement (+) or disagreement (−) between bacteriological findings and—	
	Pullorin R	Pullorin S	Rapid whole-blood agglutination test	Typical lesions noted	*Salmonella pullorum* isolated	Pullorin test	Rapid whole-blood agglutination test
30	Negative		Positive	No	Yes	−	+
414	do		Negative	No	No	+	+
13	Positive		do	Yes	Yes	+	−
70	do		do	No	No	−	+
287	do		do	No	No	−	+
172	do		do	No	No	−	+
422	do		do	No	No	−	+
74	Negative		do	Yes	Yes	−	+
10	do		do	Yes	Yes	−	+
90	do		do	Yes	Yes	−	+
148		Positive	do	Suspicious	No	−	+
101		do	do	do	No	−	+
191		do	do	No	No	−	+
135		do	do	Suspicious	No	−	+
230		Negative	Positive	Yes	Yes	−	+

TABLE 3.—*Comparison of results obtained from commercial pullorins no. 1, no. 2, and no. 3, and the rapid whole-blood and tube agglutination tests, based on their agreement or disagreement with bacteriological findings*

PULLORIN NO. 1

Fowl no.	Reaction of bird to—		Post-mortem findings					Agreement (+) or disagreement (−) between bacteriological findings and—	
	Pullorin test	Rapid whole-blood agglutination test	Tube agglutination test with blood-serum dilutions of—			Typical lesions noted	*Salmonella pullorum* isolated	Pullorin test	Rapid whole-blood agglutination test
			1:25	1:50	1:100				
95	Negative	Positive	Positive	Positive	Positive	No	Yes	−	+
76	do	do	do	do	do	No	No	+	−
83	do	do	do	do	do	No	No	+	−
451	do	do	do	do	do	Yes	Yes	−	+
107	Positive	Negative	Partial	Partial	Partial	No	No	−	+
57	Negative	Positive	Positive	Positive	Positive	No	No	+	−
25	do	do	Slight	Partial	do	No	Yes	−	+
140	do	do	Positive	Positive	Partial	No	No	+	−
82	do	do	do	do	Positive	No	Yes	−	+

PULLORIN NO. 2

2760	Positive	Positive	Positive	Positive	Partial	No	No	−	−
2766	Negative	do	do	Negative	Negative	No	No	+	−
6308	Positive	do	do	do	do	No	Yes	+	+
9182	do	do	do	Positive	Positive	Yes	Yes	+	+
6332	Negative	do	do	do	Negative	No	Yes	−	+
2756	Positive	do	do	do	Positive	No	No	−	−
2747	Negative	do	do	do	do	No	Yes	−	+
2730	Positive	do	do	do	Negative	Yes	Yes	+	+
6338	Negative	do	do	do	Positive	Yes	Yes	−	+
6376	Positive	do	do	do	do	Yes	Yes	+	+
2759	Negative	do	do	do	Slight	No	No	+	−
6386	Positive	do	do	do	Positive	Yes	Yes	+	+

PULLORIN NO. 3

170	Negative	Positive	Positive	Positive	Partial	Yes	Yes	−	+
123	do	do	do	do	Positive	No	No	+	−
164	do	do	do	do	do	Yes	Yes	−	+
177	do	do	do	do	do	Yes	Yes	−	+
175	do	do	do	do	Partial	Yes	Yes	−	+
966	do	do	do	do	do	Suspicious	Yes	−	+
903	do	do	do	Partial	Positive	do	Yes	−	+
181	do	do	do	Positive	do	Yes	Yes	−	+
967	do	do	do	do	do	Yes	Yes	−	+
159	do	do	do	do	do	Yes	Yes	−	+
982	do	do	do	do	do	No	Yes	−	+
149	do	do	do	do	Partial	No	Yes	−	+
169*	Positive	Negative				No	No	−	+
124	do	do	Negative	Negative	Negative	No	No	−	+
171	do	do	do	do	do	No	No	−	+
156	do	do	do	do	do	No	No	−	+

* This bird was found dead; consequently no tube agglutination test was made.

TABLE 4.—*Summary of agreements between the diagnostic test reactions, bacteriological findings, and results of the tube agglutination test used post mortem*

Pullorin used	Fowls examined post mortem	Agreement between pullorin test and—		Agreement between rapid whole-blood agglutination test and—	
		Bacteriological findings	Tube agglutination test	Bacteriological findings	Tube agglutination test
Experimental:	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
R	10	20.0		90.0	
S	5	0.0		100.0	
Commercial:					
1	9	45.5	0.0	55.5	100.0
2	12	58.3	58.3	66.7	83.3
3	16	6.3	0	93.8	100.0
Total or average	52	26.0	19.4	81.2	94.4

DISCUSSION

The significant results in tables 2 and 3 and summarized in table 4 are the percentages of agreement between the various tests and the bacteriological findings.

In tables 2 and 3, particular interest centers on the results with commercial pullorum no. 1. The birds used were chiefly pullets. Eight of the nine cases examined post mortem showed no macroscopic lesions of pullorum disease, and *Salmonella pullorum* was isolated from only 4 of the 9 birds. With young fowls it is more difficult to harmonize the serological and bacteriological results than with more mature ones. The corroborative evidence of the tube test in this group gives support to the inference that pullorum infection, though undiscovered in some birds, probably lurked in their bodies.

As already noted, macroscopic lesions of pullorum disease in reactors are sometimes lacking, particularly in young fowls, even when the infection may be demonstrable by bacteriological procedure. Therefore a diagnosis eliminating pullorum disease on the basis of no lesions cannot be made with assurance.

In table 4 there is seen to be excellent average agreement, 94.4 percent, between the rapid whole-blood agglutination test and the tube test, thus supporting the reliability of the rapid test. There is also good agreement, 81.2 percent, between the rapid whole-blood agglutination test and the bacteriological findings. The results obtained by the pullorin test show a much lower percentage of agreement with the bacteriological findings and the tube agglutination test. The evidence thus obtained indicates that the rapid whole-blood test is a more reliable diagnostic agent for pullorum disease than the pullorin test.

SUMMARY AND CONCLUSIONS

Each of four commercial poultry flocks was tested for pullorum disease by the stained-antigen, rapid whole-blood agglutination test and the pullorin test. In addition, the tube agglutination test was used post mortem on the serum of birds from three of the flocks, as a check on the reliability of the rapid whole-blood method. Specimen birds were obtained from these flocks, examined post mortem, studied

bacteriologically, and the findings compared with the test findings. In every group the agreement between the rapid whole-blood agglutination test and the bacteriological findings was more favorable than that between the pullorin test and the bacteriological findings. The results of the tube agglutination method, which is of recognized dependability, supported the reliability of the rapid whole-blood agglutination test.

Although the experiments here recorded are of limited scope, the evidence obtained is in accord with the views expressed by other workers, that the pullorin test is not so satisfactory a means of diagnosing pullorum disease as the rapid whole-blood agglutination test.

THE DISTRIBUTION AND CONDITION OF NITROGEN IN THREE HORIZONS OF A DIFFERENTIALLY FERTILIZED HAGERSTOWN CLAY LOAM SOIL PLANTED TO APPLE TREES IN METAL CYLINDERS[1]

By WALTER THOMAS

Professor of phytochemistry, Pennsylvania Agricultural Experiment Station

INTRODUCTION

In a recent paper (*15*) [2] the writer reported the utilization and recovery of nitrogen, phosphorus, and potassium by apple trees grown in metal cylinders for a period of 6½ years. These trees received (each spring) during the last 3 years of growth different combinations of the pure salts sodium nitrate, monocalcium phosphate, and potassium sulphate. It was shown that the ratio in which nitrogen (as N), phosphorus (as P_2O_5), and potassium (as K_2O) were absorbed from the added salts ($NaNO_3$, $CaH_4(PO_4)_2$, and K_2SO_4) by those trees optimum with respect to growth and reproduction, that is, the trees receiving the NPK and NP treatments, was 3:0.3:1.5 as compared with a 3:8:4 ratio actually applied. This latter ratio was based on the current orchard practice. The great divergence between these ratios indicated the need for information as to the condition of the added nitrogen, phosphorus, and potassium in the soil in order to determine the extent to which these "theoretical" quantities and ratios should be modified as a result of the changes produced by the interaction of the added salts with the soil. The present investigation reports the status of the nitrogen in the three soil horizons in the cylinders from which the trees referred to above were removed.

METHODS OF EXPERIMENTATION

Inasmuch as the detailed plan of this experiment has been reported elsewhere (*3, 14, 16*) a brief outline only is necessary.

The soil, the analysis of which has been recorded (*11*), was formed in place by the weathering of limestone to the lower Silurian formations, and is of Trenton origin. The excavation was made on a strip of land 110 by 11 feet adjacent to the college experimental orchard. The history of this land indicates that except for the droppings of cattle no dressings of fertilizer had ever been applied. It may, therefore, be described as a virgin forest soil. The mechanical analysis (*11*) suggests that the surface soil consists of a heavy silt loam underlain by a clay loam which becomes heavier in texture as the depth increases.

The soil was excavated from this strip by a scoop shovel. The layers from each of three horizons, viz, surface (0 to 7 inches), subsurface (7 to 21 inches), and subsoil (21 to 53 inches), were kept separate and each was thoroughly mixed and weighed. The total

[1] Received for publication Nov. 4, 1933; issued July 1934. Technical Paper no. 613 of the Pennsylvania Agricultural Experiment Station. Presented before the American Society for Horticultural Science at the Boston (1933-34) meeting of the American Association for the Advancement of Science.

[2] Reference is made by number (italic) to Literature Cited, p. 856.

weight of the horizons was 54,180, 109,200, and 249,000 pounds, respectively. Inasmuch as there were 42 cylinders, an equal distribution (by weight) of each layer among the cylinders would require the following quantities of soil to be added to each of the cylinders: Subsoil, 5,930 pounds; subsurface soil, 2,600 pounds; and surface soil, 1,290 pounds. This equal distribution was effected by ascertaining the weight of each of the respective horizons required to fill a steel wheelbarrow similar to those used in highway construction work. Such wheelbarrows were used in filling the cylinders. Following the addition of each wheelbarrow load, uniformity in density of the soil was secured by means of heavy wooden mallets fitted with 3-inch cast-iron pipe handles 5 feet in length. The process of filling the cylinders was completed in the spring of 1920. Uniformity with respect to the nitrogen, phosphorus, and potassium content of the soil in the cylinders at this stage was established by analysis. The mean of 30 determinations for total nitrogen in the original soil is given in the first line of table 1. Any departure from these values greater than the error of analysis (±0.0001 percent) must be attributed to causes resulting from differences in treatment.

The trees were planted in the spring of 1922. Up to the spring of 1924 the system of culture was similar in all cylinders. This consisted of green manuring with buckwheat and rye principally. In May 1924 half the cylinders were seeded with a mixture of Kentucky bluegrass, *Poa pratensis* L., and timothy, *Phleum pratense* L. These cylinders are designated "cylinders under sod." In the remaining half of the cylinders a tillage system was adopted. These latter cylinders are designated "cylinders under cultivation." A distinction must be noted with respect to the additions of nitrogen from 1925 until the end of the experiment in 1927. During these last 3 years of the experiment the cylinders under cultivation received 15.9 grams more nitrogen than the cylinders under sod. The reason for this is that it was then considered necessary to add equal amounts of organic matter to all the cylinders under cultivation. This was accomplished by growing rye outside the cylinders. For further details the paper by Anthony and Clarke (*3, p. 251*) should be consulted. All trees were allowed to grow without the addition of any mineral fertilizer until the spring of 1925, at which time differential treatment with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate was commenced. It is important to note that the conditions of this experiment preclude any erosion by water and practically none by wind.

The sodium nitrate was added according to the following schedule:

	Grams
April 18, 1925	906
May 3, 1926	45
June 7, 1926	453
June 20, 1926	408
May 5, 1927	337
May 18, 1927	338
June 10, 1927	337
Total	2,824

This total of 2,824 g of sodium nitrate is equivalent to 465.5 grams of elemental nitrogen.

During the period from September 20 to 28, 1927, the trees were dup up and soil samples representative of the three horizons were taken, by the method of successive quartering, from each of the cylinders from which the trees had been removed. These samples were dried at 75° C. and then sieved through a 1-millimeter sieve (*4*) and stored in glass jars in the dark. Analyses of the trees have already been reported (*14*).

Total nitrogen was determined by the Kjeldahl-Gunning method to include the nitrogen of nitrates, a 15-g charge being used for the surface soil and 30-g for the subsurface and subsoil (*4*). Nitric nitrogen was determined by the Devarda alloy method, a 200-g charge being used (*1*). The analytical data in the tables are the means of closely agreeing triplicate determinations. The quantities of ammonia nitrogen and nitrous nitrogen in all horizons were insignificant. All calculations are made on a moisture-free basis.

EXPERIMENTAL DATA

Table 1 gives the percentage and absolute amounts of total nitrogen in each of the three soil horizons; that is, the 0 to 7 inch, the 7 to 21 inch, and the 21 to 53 inch.

TABLE 1.—*Percentages and absolute amounts of total nitrogen in the respective horizons before the trees were planted and at the end of the experiment*

Treatment	Total nitrogen			Absolute amount total nitrogen			
	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Total (0–53 inches)
	Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	*Grams*
Soil before trees were planted	0.08650	0.05003	0.03523	506.1	589.7	946.8	2,042.6
Sod:							
Check	.08219	.04600	.03400	477.9	542.5	914.5	1,934.9
PK	.08200	.04700	.03498	479.8	554.3	941.4	1,975.5
NPK	.08200	.05160	.03900	478.6	613.2	1,049.0	2,140.8
NP	.08180	.05250	.03900	478.6	619.1	1,049.0	2,146.7
NK	.08200	.05500	.04040	479.8	648.6	1,083.0	2,211.4
N	.08200	.05200	.04000	479.8	625.0	1,075.9	2,180.7
Cultivation:							
Check	.07110	.05100	.03445	415.9	601.5	926.6	1,944.0
PK	.07485	.05000	.03571	436.4	590.4	960.5	1,987.3
NPK	.07800	.05290	.04310	456.4	620.8	1,146.6	2,223.8
NP	.07900	.05230	.04250	462.2	614.4	1,142.9	2,219.5
NK	.07820	.05500	.04350	457.6	648.8	1,170.1	2,276.5
N	.07890	.05200	.04200	461.6	620.2	1,131.9	2,213.7

Table 2 gives the percentage and absolute amounts of nitric nitrogen and of nonnitric nitrogen. The latter values were obtained by difference between the total nitrogen and the nitric nitrogen.

TABLE 2.—*Percentage and absolute amounts of nitric and nonnitric nitrogen in the respective horizons before trees were planted and at the end of the experiment*

Treatment	Nitric nitrogen			Absolute amount nitric nitrogen			
	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Total (0–53 inches)
	Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	*Grams*
Soil before trees were planted	0.00170	0.00053	0.00023	9.9	6.3	6.2	22.4
Sod:							
Check	.00069	.00080	.00060	4.0	9.4	16.1	29.5
PK	.00070	.00100	.00066	4.1	11.8	17.7	33.6
NPK	.00220	.00760	.00450	12.9	89.6	121.0	223.5
NP	.00200	.00720	.00500	11.7	84.9	133.5	230.1
NK	.00290	.00900	.00560	16.9	106.1	147.0	270.0
N	.00240	.00820	.00550	14.0	96.7	147.9	258.6
Cultivation:							
Check	.00110	.00100	.00065	6.4	11.8	17.5	35.7
PK	.00085	.00120	.00071	3.5	14.1	19.1	36.7
NPK	.00210	.00640	.00410	12.3	72.4	111.0	195.7
NP	.00200	.00610	.00460	11.7	69.6	123.5	204.8
NK	.00270	.00690	.00590	15.8	81.5	158.7	256.0
N	.00290	.00760	.00500	16.9	89.5	136.7	243.1

Treatment	Nonnitric nitrogen			Absolute amount nonnitric nitrogen			
	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Surface (0–7 inches)	Subsurface (7–21 inches)	Subsoil (21–53 inches)	Total (0–53 inches)
	Percent	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	*Grams*
Soil before trees were planted	0.0848	0.0495	0.0350	496.2	583.4	940.6	2,020.2
Sod:							
Check	.0815	.0452	.0334	473.9	533.1	898.4	1,905.4
PK	.0813	.0460	.0343	475.7	542.5	923.7	1,941.9
NPK	.0798	.0444	.0345	468.9	523.6	928.0	1,918.5
NP	.0798	.0453	.0340	466.9	534.2	915.5	1,916.6
NK	.0791	.0460	.0348	462.9	542.5	936.0	1,941.4
N	.0796	.0448	.0345	465.8	528.3	928.0	1,922.1
Cultivation:							
Check	.0700	.0500	.0338	409.5	589.7	909.1	1,908.3
PK	.0740	.0488	.0350	432.9	576.2	941.4	1,950.5
NPK	.0759	.0465	.0390	444.1	548.4	1,035.6	2,028.1
NP	.0770	.0462	.0379	450.5	544.8	1,019.4	2,014.7
NK	.0755	.0481	.0376	441.8	567.3	1,011.4	2,020.5
N	.0760	.0450	.0370	444.7	530.7	995.2	1,970.6

DISCUSSION OF DATA

TOTAL NITROGEN

The data in table 1 indicate that at the end of the experiment the total nitrogen content of the surface soil in all cylinders under sod was slightly greater than that in cylinders under cultivation, although, as has already been pointed out, the cylinders under cultivation had received 15.9 grams more nitrogen than the corresponding cylinders under sod. The differences between the total nitrogen content of corresponding cylinders under the two systems in the respective horizons are shown in table 3.

The data in table 3 do not take into account the nitrogen removed by the trees and, in sod, by the grass also. The disappearance of nitrogen (as total nitrogen) when the amounts removed by the trees are taken into account is shown in table 4. This disappearance is called by some investigators "the apparent loss of nitrogen."

TABLE 3.—*Difference* [a] *(in grams) between the amounts of total nitrogen present in the 3 horizons of the 2 systems*

Horizon	Check	PK	NPK	NP	NK	N
Surface (0–7 inches)	+62.0	+43.4	+23.4	+16.4	+22.2	+18.2
Subsurface (7–21 inches)	−59.0	−36.1	−7.6	+4.7	0	+4.8
Subsoil (21–53 inches)	−12.1	−19.1	−97.6	−93.9	−87.1	−56.0
Total (0–53 inches)	−9.1	−11.8	−81.8	−72.8	−64.9	−33.0

[a] The sign indicates the amount in grams by which the total nitrogen under sod is greater than (+) or less than (−) under cultivation.

TABLE 4.—*Nitrogen disappearance (grams) calculated on the total nitrogen of the soil in the whole layer (0 to 53 inches) at the end of the experiment*

Item	Sod					
	Check	PK	NPK	NP	NK	N
(1) Amount N present in soil before experiment	2,042.6	2,042.6	2,042.6	2,042.6	2,042.6	2,042.6
(2) Amount N added in $NaNO_3$ (=465.0 g)+seeds (=2.5 g)	2.5	2.5	467.5	467.5	467.5	467.5
(3) Amount N from (1)+(2)	2,045.1	2,045.1	2,510.1	2,510.1	2,510.1	2,510.1
(4) Amount N found	1,934.9	1,975.5	2,142.0	2,146.7	2,211.4	2,180.7
(5) Loss of N from soil	−110.2	−69.6	−368.1	−363.4	−298.7	−329.4
(6) Total amount N absorbed by trees during growth (tops and roots)	36.5	56.8	201.4	190.4	133.4	124.3
(7) Disappearance of N by leaching and possibly as gaseous N during the 6½ years of the experiment	−73.7	−12.8	−166.7	−173.0	−165.3	−205.1

Item	Cultivation					
	Check	PK	NPK	NP	NK	N
(1) Amount N present in soil before experiment	2,042.6	2,042.6	2,042.6	2,042.6	2,042.6	2,042.6
(2) Amount N added in $NaNO_3$ (=465.0 g)+seeds (=2.5 g)	19.4	19.4	483.4	483.4	483.4	483.4
(3) Amount N from (1)+(2)	2,062.0	2,062.0	2,526.0	2,526.0	2,526.0	2,526.0
(4) Amount N found	1,944.0	1,987.3	2,223.8	2,219.5	2,276.5	2,213.7
(5) Loss of N from soil	−118.0	−74.7	−302.2	−306.5	−249.5	−312.3
(6) Total amount N absorbed by trees during growth (tops and roots)	53.5	63.4	180.9	170.3	131.8	121.3
(7) Disappearance of N by leaching and possibly as gaseous N during the 6½ years of the experiment	−64.5	−11.3	−121.3	−136.2	−117.7	−191.0

Considering the whole depth 0 to 53 inches, the losses from the nitrogen-treated cylinders are greater under sod than under cultivation. The amounts by which the losses (in grams) under the former system exceed those under the latter are: NPK, 45.4; NP, 36.8; NK, 47.6; and N, 14.1 These differences appear to be related to the accretion of nonnitric nitrogen in the subsoil of the cylinders under cultivation. The net result is a gain in nonnitric nitrogen when calculated on the whole depth (0 to 53 inches) in the treated cylinders under cultivation as compared with those under sod. This point is discussed later.

Line 7 of table 4 gives the losses by leaching and possibly as gaseous nitrogen (i.e., the so-called "nitrogen balance") for the whole soil layer (0 to 53 inches). The same data calculated for the surface 0 to 7 inches and subsurface 0 to 21 inches only are shown in table 5.

TABLE 5.—*Nitrogen balance (in grams) calculated to less than full depth*

Horizon	Check	PK	NPK	NP	NK	N
0 to 7 inches:						
Under sod	+5.8	+34.0	−292.4	−304.6	−360.4	−309.5
Under cultivation	−56.1	−25.7	−352.2	−357.0	−400.1	−406.6
0 to 21 inches:						
Under sod	−41.4	−7.4	−268.9	−275.2	−301.5	−334.2
Under cultivation	−44.3	−25.0	−321.1	−332.3	−341.0	−376.1

The nitrogen balance is seen to vary with the depth of soil upon which the calculations are based. In the 0- to 7-inch layer, a nitrogen gain is indicated in the cylinders under sod to which no mineral nitrogen was added. But if the calculations are based on the 0- to 21-inch layer or on the whole depth, 0- to 53-inch layer, losses of nitrogen are definitely established. The larger losses shown in the nitrated cylinders in the 0- to 7-inch and 0- to 21-inch layers as compared with the 0- to 53-inch layer appear to be only an expression of the fact that the quantity of nitrates (nitric nitrogen) becomes greater with depth.

More information with respect to the status of the nitrogen is obtained by considering the nitric and nonnitric fractions of the total nitrogen separately. These are shown in table 2.

NITRIC NITROGEN (NITRATES)

Mobility of Added Nitric Nitrogen

Table 2 shows that the nitric nitrogen calculated on a percentage basis, i.e., the concentration of nitrates, is greater in the subsurface than in either of the other horizons. The absolute amount of nitric nitrogen, however, is greater in the subsoil in all cases. The last application of $NaNO_3$ (167 grams nitrogen) was made in the spring of 1927, 4½ months before the trees were removed. Presumably, therefore, this increased concentration of nitric nitrogen in the subsurface is merely an expression of the movement of nitrogen as nitric nitrogen from this last application, and when taken in conjunction with the data for the nitric nitrogen in the check and PK cylinders, suggests that the greater part of the last application was still in the 7- to 21-inch layer at the conclusion of the experiment.

Nitric Nitrogen Under Sod and Cultivation

It will be recalled that each of the cylinders under cultivation received 15.9 grams more nitrogen in the form of rye cover crop than the cylinders under sod. Now the concentration of nitric nitrogen in the whole depth (0 to 53 inches) of the check cylinder under cultivation is 35.7 grams and that of the cylinder under sod is 29.5 grams as compared with 22.4 grams in the original soil. However, in the cylinders which received mineral nitrogen in addition to that introduced by the green manures (the NPK, NP, NK, and N cylinders) the concentration of nitric nitrogen in the whole depth is much greater in all cases in the cylinders under sod. This may be only another expression of the difference in the status of the soil nitrogen in the three horizons under sod and cultivation previously referred to in the discussion of the disappearance of nitrogen (as total N) by leaching and possibly as gaseous nitrogen. This will be brought out more clearly in the discussion of the nonnitric nitrogen fraction.

An Inventory of Nitric Nitrogen

A more complete picture of the status of the nitrates may be obtained from the inventory of nitric nitrogen shown in table 6, in which account has been taken of the nitric nitrogen equivalent to that absorbed by the trees under the different treatments from the added $NaNO_3$.

TABLE 6.—*Inventory of nitric nitrogen (in grains) at end of experiment (0 to 53 inches)*

Item	Sod					
	Check	PK	NPK	NP	NK	N
(1) N added to each cylinder in the form of $NaNO_3$	0	0	465.5	465.5	465.5	465.5
(2) N absorbed by each tree during growth and also (in sod) by the grass	36.5	56.8	201.4	190.4	133.4	124.3
(3) N absorbed by each tree from added $NaNO_3$	0	0	132.7	132.4	91.9	83.6
(4) Theoretical amount of N expected in soil			332.8	333.1	373.6	381.9
(5) N found	29.5	33.6	223.5	230.1	270.0	258.6
(6) Disappearance of nitric N during the 6½ years of the experiment			109.3	103.0	103.6	123.3

Item	Cultivation					
	Check	PK	NPK	NP	NK	N
(1) N added to each cylinder in the form of $NaNO_3$	0	0	465.5	465.5	465.5	465.5
(2) N absorbed by each tree during growth and also (in sod) by the grass	53.5	63.4	180.9	170.3	131.8	121.3
(3) N absorbed by each tree from added $NaNO_3$	0	0	117.5	93.2	78.3	67.8
(4) Theoretical amount of N expected in soil			348.0	372.3	387.2	397.7
(5) N found	35.7	37.0	195.7	204.8	256.0	243.1
(6) Disappearance of nitric N during the 6½ years of the experiment			152.3	167.5	131.2	154.6

The nitric nitrogen absorbed by the trees (table 6, line 3) was obtained in a manner similar to that described in an earlier paper (*15, pp. 570–573*). The values in line 3 represent the difference between the amount of nitrogen absorbed by a tree which received additions of another element (or other elements) than nitrogen and a tree from which nitrogen was omitted. The values so obtained may not be mathematically exact, inasmuch as the Wirkungswert (effect factor) of an element may not be the same in the presence of another factor or factors as when that factor operates alone. Nevertheless, there are numerous experiments that lend support to Mitscherlich's contention (*7*) that the Wirkungswert of an element may be fairly constant, especially under the controlled conditions of such an experiment as the present one. The method is believed to be sufficiently accurate to bring out more clearly any characteristic differences in the status of the nitric nitrogen of the respective treatments and especially with respect to differences between the two cultural systems. The procedure adopted may be more readily understood from the following algebraical analysis:

Let a = amount of nitrogen present in the soil of each cylinder before the trees were planted.

Let x = amount of nitrogen added to each cylinder in rain and snowfall.
Let y = amount of nitrogen added to each cylinder under cultivation in the form of organic matter (buckwheat and rye).
Let z = the total amount of sodium nitrate added to each of the "nitrated" cylinders.

Now the nitrogen-treated trees have obtained the nitrogen absorbed by them from all of the foregoing sources, and the trees which did not receive mineral nitrogen ($NaNO_3$) additions absorbed nitrogen from all of these sources except z.

For greater simplicity and clarity, let us first of all consider the absorption of nitrogen from only two of the trees on which chemical analyses were made; namely, the NPK and the PK trees, both under the tillage system.

The amount of nitrogen absorbed by the NPK tree during the whole period of its growth will be some fraction of $a+x+y+z$. Let this fraction be designated k $(a+x+y+z)$. Similarly, the amount of nitrogen absorbed by the PK tree during the whole period of its growth will be some fraction of $(a+x+y)$, which will be designated $k'(a+x+y)$.

$$\text{Let } \frac{1}{s}=k(a+x+y+z) \quad (1)$$

$$\text{Let } \frac{1}{r}=k'(a+x+y) \quad (2)$$

Then, by subtraction

$$\frac{1}{s}-\frac{1}{r}=k(a+x+y+z)-k'(a+x+y) \quad (3)$$

Now, if $k(a+x+y)$ is equal to or very nearly equal to $k'(a+x+y)$,

$$\text{then, from (3), } \frac{1}{s}-\frac{1}{r}=kz \quad (4)$$

or, expressed in words, the fraction of the nitrogen added to the NPK tree in the form of sodium nitrate is obtained by difference between the total amount of nitrogen absorbed by that tree and that absorbed by the PK tree.

In the present experiment the trees were grown without mineral salt additions for the first 4 years. The factor z then of equation (1) does not enter into the picture during this period.

For the purpose of the present analysis the difference between the quantities $k(a+x+y)$ must be very small as compared with the quantity kz. In further support of this contention may be cited the mathematical analysis given by the writer in an earlier paper (*13*).

The amounts of nitrogen applied as sodium nitrate were 149 g in 1925, 149 g in 1926, and 167 g in 1927, a total of 465 g. A comparison of these quantities of added nitrogen with the quantities actually present (table 6, line 5) shows that all of the "nitrated" cylinders contained at the end of the experiment more than enough nitrates to account for the amount (167 g) added the last year of the experiment, 4½ months before the samples were taken in the fall of 1927, and in addition a considerable portion of the nitrogen added in the second application in the spring of 1926. The total precipitation during the period between one application and the next was: May 18, 1925, to May 2, 1926, 31.8 inches; May 3, 1926,

to May 4, 1927, 44 inches; May 5, 1927, to September 20, 1927, 18.6 inches. In addition, 2 inches of artificial watering was applied in May 1926 and 1 inch in August 1927.

The Rothamsted experiments (*9*) on the losses of nitrogen in the drainage waters from a plot of arable land kept free of vegetation since 1870, which received no artificial additions of nitrogen, are frequently cited in support of the view that nitrates are readily leached from soils. At the end of 47 years the amounts of nitric nitrogen found in the drainage waters were equal to the total losses of nitrogen from the soil. The rate of loss was equal to 40 pounds per annum per acre in the earlier years and below 25 pounds per annum per acre in the later years.

In some soil types in Tennessee (*8*), however, when nitrogen was applied as sodium nitrate to lysimeters kept bare of vegetation, the leaching (outgo) of nitrogen in 2½ years was as low as 34 percent. Mooers and MacIntire attribute this relatively small loss to the influence of the heavy clay subsoil into which the nitrate ion passes as magnesium and calcium nitrates through base exchange.[3]

The investigations of De Sornay (*10*) also indicate that nitrates may remain in the soil available to plants for long periods, moving upward or downward according to moisture conditions. The upward capillary attraction was found to be much more rapid than the downward displacement by rain. De Sornay reports that Demolon and Brouet at Aisne found, in an uncropped, light, sandy garden soil, that after a rainfall of 9.8 inches during a period of 2 months more than one-half of the added sodium nitrate remained in the first 8 inches.

More recently Ames (*2*) has reported that during the period 1928–30 the nitric nitrogen content of the soil under corn or soybeans never exceeded 50 pounds per acre in the surface 6⅔ inches, but in 1931, after a year of drought, the nitric nitrogen content reached 300 pounds per acre.

The problem is summed up by MacIntire [4] as follows:

> It is exceedingly difficult to make an unqualified statement as to the fate of added nitrogen. This will vary with the soil, alkalinity or acidity, climatic conditions, the amount of added nitrogen, absence or presence of growing plants and the type of these, and periodicity of rainfall, together with the very important fact of depth and type of subsoil.

In the present experiment the significant fact is that the disappearance of nitric nitrogen is greater in all the cylinders under cultivation to which mineral nitrogen was added than under the corresponding cylinders under sod.

NONNITRIC NITROGEN

The nonnitric nitrogen consists of (1) nitrogenous organic material potentially "available" but not yet decomposed; (2) the humus nitrogen characterized by marked stability; (3) the nitrogen synthesized by the micro-organisms; (4) ammonia nitrogen absorbed by the colloidal soil complex.

The apparent gain or loss in integral numbers with respect to the nonnitric nitrogen is given in table 7. The quantities of nitrogen shown in line 4, that is, the amount of nitrogen absorbed by the trees from sources other than the nitrogen added as $NaNO_3$, were obtained

[3] MacIntire, W. H. Private communication.
[4] MacIntire, W. H. See footnote 3.

by using the quantities of nitrogen absorbed by corresponding treatments to which no nitrogen was added. For example, the amount of nitrogen absorbed by the NPK tree in sod from sources other than that equivalent to the added $NaNO_3$ was obtained by difference between this quantity and that absorbed by the PK tree also growing in sod. For reasons already given these values have no pretention to mathematical exactness. But in the present analysis they serve for all practical purposes to furnish a picture of the changes in nonnitric nitrogen of the original soil as a result of the various treatments under the two culture systems.

TABLE 7.—*Gain or loss (grams) of nonnitric nitrogen from whole depth of soil (0 to 53 inches) by the end of the experiment*

Item	Sod					
	Check	PK	NPK	NP	NK	N
(1) Nonnitric N before experiment	2,020.2	2,020.2	2,020.2	2,020.2	2,020.2	2,020.2
(2) Nonnitric N at end of experiment	1,905.4	1,941.9	1,918.5	1,916.6	1,941.4	1,922.1
(3) Actual loss or gain by soil	−114.8	−78.3	−101.7	−103.6	−78.8	−98.1
(4) N absorbed per tree from sources other than the added $NaNO_3$	36.5	57.0	57.0	49.9	37.4	37.4
(5) Apparent total loss or gain during the 6½ years of the experiment	−78.3	−21.3	−44.7	−53.7	−41.4	−60.7

Item	Cultivation					
	Check	PK	NPK	NP	NK	N
(1) Nonnitric N before experiment	2,020.2	2,020.2	2,020.2	2,020.2	2,020.2	2,020.2
(2) Nonnitric N at end of experiment	1,908.3	1,950.5	2,028.1	2,014.7	2,020.5	1,970.6
(3) Actual loss or gain by soil	−111.9	−69.7	+7.9	−5.5	+.3	−49.6
(4) N absorbed per tree from sources other than the added $NaNO_3$	53.5	63.4	63.4	77.1	53.5	53.5
(5) Apparent total loss or gain during the 6½ years of the experiment.	−58.4	−6.3	+71.3	+71.6	+53.8	+3.9

In both culture systems there is a disappearance of nonnitric nitrogen in the cylinders which received no mineral nitrogen additions (check and PK cylinders), the loss being greater under sod. But, whereas relatively large accretions of nonnitric nitrogen have occurred in the NPK, NP, NK, and N cylinders under cultivation, losses of nonnitric nitrogen have occurred from the corresponding cylinders under sod. These differences are much greater than can be accounted for by sampling or analytical errors. Although uniformity with respect to content of nitrogen, phosphorus, and potassium was established in the cylinders before the experiment began, there may have existed differences in respect to the physical condition that would preclude a definite and unqualified interpretation of the differences existing with respect to the condition of the nitrogen under sod and cultivation. Three explanations may be advanced:

(1) Assimilation of nitrogen added as $NaNO_3$ by micro-organisms. But the difficulty lies in explaining why assimilation should have occurred in the cylinders under cultivation and not in those under sod. Carbon dioxide accumulation under grass (*12*) may be the differential factor. Some nitrogen would appear to have been brought up and immobilized in the surface soil by the grass roots in the sod system, inasmuch as the quantity of nitrogen (as total and nonnitric nitrogen)

of the surface soil is higher in all cases under grass than under cultivation. But the results for the whole depth (0 to 53 inches) show that this explanation is insufficient to account for the entire difference in the amount of nitrogen in corresponding cylinders in the two systems.

(2) The peptization of nitrogenous organic material by $NaNO_3$ in the first horizon and subsequent leaching. Hardpan formation was particularly noticeable in the nitrogen-treated cylinders under cultivation. Cracks and fissures, therefore, may have assisted the downward movement.

(3) The greater root system under cultivation. Except in one tree (NPK) the root systems were larger in the trees grown under cultivation. The weights of the root systems are given in table 8.

TABLE 8.—*Weights in grams of the respective root systems in soils in sod and under cultivation*

Condition	Check	PK	NPK	NP	NK	N
Sod	9,950	8,325	13,040	12,730	11,090	10,366
Cultivation	10,870	10,695	12,395	13,075	11,700	12,275

The higher nitrogen content of the soil under cultivation may have arisen from sloughed-off portions of fibrous roots that might have been incorporated in the soil in the process of preparation for analysis.

The extensive literature of the problem of the mineralization of nitrogen in the soil has been reviewed by Lemoigne (*5*), Lyon (*6*), and Waksman (*17*). The results presented in this paper suggest the desirability of further investigation of the problem.

SUMMARY

The distribution of total nitrogen and also of the nitric and nonnitric fractions in three horizons, viz, 0 to 7 inches, 7 to 21 inches, and 21 to 53 inches, of a Hagerstown clay loam soil contained in cylinders planted to apple trees and treated with different combinations of sodium nitrate, monocalcium phosphate, and potassium sulphate are given in percentage and in absolute amounts.

In all treatments the total nitrogen of the surface soil under sod was somewhat greater than under cultivation. In the subsurface the differences in total nitrogen were small except in the check cylinders under sod, in which it was less than under tillage. In all treatments the total nitrogen of the subsoil was greater in the cylinders under cultivation than in the corresponding cylinders under sod. For the whole depth (0 to 53 inches), the total nitrogen at the end of the experiment was greater in all cylinders under cultivation than in those under sod.

The disappearance of nitrogen (as total nitrogen) by leaching, and possibly as gaseous nitrogen, was greater under sod than under cultivation in all cases.

The movement of nitric nitrogen is discussed. It is concluded that leaching of nitrates from this heavy soil was not very rapid.

The disappearance of nitric nitrogen, when account has been taken of the nitric nitrogen absorbed by the trees equivalent to that added as $NaNO_3$, was greater in all nitrated cylinders under cultivation than

in corresponding cylinders under sod. This difference is accounted for by an accretion of nitrogen as nonnitric nitrogen in the subsoil under cultivation but not in that under sod.

Results with respect to nitrogen gains or losses based on the soil to a depth of 53 inches are very different from those based on the 0- to 7-inch or 0- to 21-inch depths.

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